Investigating life-history polymorphism: Modelling mites

Nils B. Koesters

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ABSTRACT

The thesis presents research on the life-history polymorphism in the mite *Sancassania berlesei*. Males of this species are andropolymorphic: there are two distinct male phenotypes. One, the fighter, develops a third thickened leg pair, with which it kills off other fighters and males which do not exhibit a third thickened leg pair, the non-fighters.

A review of the life-history of *S. berlesei* is given, focussing on its general biology, diet, dispersal and mating behaviour. This is followed by a review of the andropolymorphism, and the current understanding of the mechanisms underlying it. The major conclusions from the experimental work presented in this thesis are that fighters primarily develop at low population densities; though the proportion of males becoming fighters at any given density may change over time. This change is likely to be due to condition-dependence. Data is presented to illuminate these matters and a model is developed linking fighter development to the costs of being a fighter (in terms of survival) and the benefits of being a fighter (in terms of fecundity).

The sex ratio in *S. berlesei* is 1:1, and there is no evidence of density or frequency-dependent deviations from this. A delay in food supply at mat-

uration delays the time of maximum fecundity of females for about seven days and lowers their overall egg output. Density-dependent effects reduce the overall daily fecundity of females in higher densities. Female survival is affected by density, food present and rearing conditions. Nearly all eggs laid by *S. berlesei* hatch regardless of the conditions. Eggs laid in very poor conditions hatched even earlier than the average time of between day three and four. At density two, animals do synchronise their frequency, when isolated together from egg stage. Poor conditions reverse female density-dependence from convex to concave with the lowest life expectancy at intermediate densities. The trade-off between survival and fecundity is the likely cause.

Amalgamating the results from the previous experiments, the influence of stochastic population dynamics on male strategy was then modelled. The results indicate that the fighter morph development rule is sensitive to the probability of low population densities arising. When low densities occur, there is a selective advantage to being a fighter. With increasing probability of lower densities, becoming a fighter is more feasible. The ESS rule changes, while in a stable high density environment a density-dependent fighter rule is never selected for. This indicates an influence of stochastic population dynamics on life-history evolution. Modelling demographic stochasticity in the fighter rule shows some buffering effect of this form of stochasticity. The fighter morph determination rule is less sensitive to environmental stochasticity with a high frequency of low densities.

Using an agent based model with diploid genetics, I show that under high densities a fighter male is less successful at passing on his genes than

a non-fighter. At a density of one male, the fighter gains no advantage to developing the fighter phenotype (as he is not competing with other males). In this case, the advantage may arise through future increases in density (such as through immigration or maturation of offspring). The density-dependent fighter development rule is then switched within the model from density-dependent to frequency-dependent, and the model indicates, that even under the frequency-dependent rule a possible ratio of fighters to non-fighters could exist. The system does not reach this state due to condition-dependence in reality.

Following on from the findings discussed above, that morph determination has a condition-dependent component, I develop an argument that relates the observed forms of morph determination (density-dependent and frequency-dependent) in three closely related species of mites via an underlying condition-dependence. It is shown that condition-dependence is likely the linking factor between frequency and density-dependence. This is shown to be possibly a rule for all species displaying polymorphism which includes physical alterations of their bodies.

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Abstract

1.) The linkages between the study of evolution and the study of diversity will be discussed.
2.) Mechanisms by which phenotypic and genetic variation are maintained in populations will be detailed.
3.) Links between intraspecific variation and speciation will be highlighted.
4.) Concentrating on polymorphism, the concept of andropolymorphism is introduced using a specific animal system, more specifically a mite species.
5.) The question addressed in this thesis is: "What maintains the polymorphism shown by the mite system?"
6.) I discuss how to assess the adaptive value of traits, contrasting optimality approaches with those based on invasibility analysis. This includes an introduction to the modelling approaches taken in this thesis.

1.1 Introduction

Understanding biodiversity and its causes is a focus of biological research (Diekmann and Doebeli, 1999; Bridle, 2000; Pachepsky et al., 2001). Biodiversity has been most generally defined as the "full variety of life on Earth" (Takacs, 1996). More specifically, the study of biodiversity is the study of the processes that create and maintain variation. It is concerned with the variety of individuals within populations, the diversity of species within communities, and the range of ecological roles within ecosystems (Takacs, 1996).

Ecologists still search for common principles that predict well known responses of biodiversity to different factors. Such factors include the number of available niches in space, productivity, area, species' body size and habitat fragmentation (Richie and Olff, 1999). The maintenance of genetic diversity within a species can also shed light on the genetic variation between species (Solé et al., 1999), as ultimately changes within a species have to take place, for new species to evolve. In extreme cases speciation can be triggered by just one changed gene. This happens in the Japanese land snail *Euhadra*, where one gene alters the chirality of the entire ontogeny. This introduces chirality constraints on mating (Ueshima and Asami, 2003).

Differences within (and between) species can be described at a genetic or phenotypic level. The genotype is the internally coded, inheritable information carried by all living organisms, the DNA. The DNA information is used as a set of instructions for building and maintaining all living creatures. These instructions (the genetic code) are found within almost all cells. They are copied at the time of cell division or reproduction and are passed from one generation to the next.

The phenotype would be defined as the physical manifestation of an organism, like cells, structures, metabolism, energy utilisation, tissues, organs, reflexes and behaviours. If a species develops several discrete phenotypes, the term polymorphism is used. In some species one sex develops several phenotypes (Gross, 1996). If the sex carrying the polymorphism is female this is called gynopolymorphism and if the polymorphic sex is male (like in the animal model system of this study) this is called andropolymorphism.

Besides the detailed description of andropolymorphism, the detailed study of the evolution of alternate male strategies has not received much attention (Gross, 1996; Kurdziel and Knowles, 2002) and has been mostly empirical (Schroeder et al., 1996; Radwan and Klimas, 2001; Cremer and Heinze, 2002; Kurdziel and Knowles, 2002). Using *Sancassania berlesei* (an andropolymorphic mite with two distinct phenotypes, a so-called "fighter" and a "non-fighter") as a model organism this study will be a detailed investigation of the costs and benefits of developing into each morph. The costs and benefits will be examined over the organisms whole life-history, based on a detailed empirical study of all aspects of *S. berlesei*'s life-history.

1.1.1 Phenotypic variation

Phenotypic plasticity is the property of a genotype to produce different phenotypes when exposed to different environments. Plasticity is therefore a description of the reaction norm of a genotype, which is the function defined in environment / phenotype space relating environmental input to phenotypic output.

form of variation	characterist.	number of phenotypes in a population	number of structural classes within an individual	example
unique phenotype	channelling	1	1	*Scathophaga stercoraria*, (Hosken et al., 2000)
phenotypic modulation	broad spectrum of expressions	depending on environment, numerous classes, normally continuous variation	depending on environment, numerous classes, normally continuous variation	*Bordetella pertussis* Whooping cough bacterium
developmental conversion	discrete expressions initiated by the environment	>=2	1	*S. berlesei*
genetic morphs	genetic specialisation	>=2	1	possibly *Rhizoglyphus robini*
multiple strategies	different kind of variations are expressed in the same individual	1	>=2	hepatitis C virus

Tab. 1.1: *Forms of phenotypic variation, after Lloyd (1984) with use of the terminology of Smith-Gill (1983).*

A wide range of forms of phenotypic variation exists. There may be a single phenotype (this is when development is said to be 'channeled') or there can be continuous or discontinuous variation (see table 1.1). In a channeled phenotype, development is buffered against a variation of the genotype and against variation of the environment (Waddington, 1952).

Developmental conversion occurs when environmental signals influence the development of an animal into different types (Lloyd, 1984). Here the development is directed in one or several discrete ways, which can bring changes in many traits of an organism. *S. berlesei*, an acarid mite, provides an example of such a developmental conversion, which is seen to be triggered by an environmental signal (Woodring, 1969; Timms et al., 1980, 1981a,b; Radwan, 1992, 1993), where it has been shown that a different phenotype develops at low population densities. Developmental conversions can also be found in the larval stages of *Nemoria arizonaria* which develop mimesis of pussy willows or twigs (Greene, 1989). Here the environmental trigger is the tannin concentration, which regulates if the caterpillars develop into mimics of pussy willows or twigs.

Between the extremes of channelling (single phenotype) and developmental conversion (phenotype is discrete and depends on the environment), organisms can show a variety of forms of phenotypic plasticity. Here the different traits of an organism can differ in the extent of a variation. This phenotypic modulation does not have to be adaptive (Smith-Gill, 1983). Variations in growth can also occur because of nutritional differences, but the response to different food levels may be itself adaptive.

A discrete phenotype can exhibit not only different morphologies but also differences in behaviour and life-history strategies.

1.1.2 Polymorphism of one sex

Polymorphism in one sex has been found in many taxa, including arthropods, fish, lizards and birds. It can show itself in differences in morphology, behaviour, physiology as well as life-history (Gadgil, 1972; Gross, 1996). Examples of the taxa displaying polymorphism in one sex are the amphipods *Jassa marmorata* and *Jassa falcata* (Borowsky, 1985; Conlan, 1989), the beetle *Onthophagus acuminatus* (Emlen, 1999), the bird *Philomachus pugnax* (Lank et al., 1995) and some species of mites (notably from the family Acaridae)(Evans et al., 1961) (See figures 1.1, 1.2, 1.3).

Andropolymorphism (a species having two or more male phenotypes) in mites can generally be found in the Astigmata (Zakhvatkin, 1959; Hughes, 1976; Timms et al., 1981a,b) (See figure 1.2).

1.2 Reasons for cause & maintenance of within species diversity

Fisher (1930) showed that traits linked to fitness would quickly be selected to fixation. Therefore, on average, the expectation is that intraspecific variation (in a constant environment) should be very low. That genetic and phenotype variation is common begs explanation. A number of potential mechanisms by which variation is maintained are discussed below.

1.2.1 Genotype x environment interactions

Phenotypic plasticity is exhibited whenever the phenotype of a gene changes predictably with changes in the environment. The phenotypic variation can be continuous or discrete. The spectrum of the phenotype across the different environments is known as a reaction norm (Johanssen, 1911; Bradshaw, 1965). It is possible to distinguish between two forms of reaction norms. In the first form, all genotypes react to the environment in a similar manner, so that along an environmental gradient there is no particular order of "fitness" of the genotypes. With the second form, the genotypes vary in the extent of their reaction to environmental gradients, so that the phenotypes change in different ways when the ranking of the genotype's fitness changes. This variation of reaction norms is called a genotype-environment interaction. As different genotypes do better in different environments, this implies that environmental variation will maintain genetic variation.

1.2.2 Mutation/selection balance and Fisher's fundamental theorem

Mutation creates diversity by introducing new alleles. If selection occurs against a deleterious allele, it will eventually be lost from the population. This can lead to a balance between selection and mutation. If a deleterious allele continues to exist, this will be due to a "balance" between mutation and selection.

The mutation/selection balance can be used to infer indirect (e.g.

pleiotropic) selection. If a deleterious allele is more frequent than its mutation/selection balance (calculated from the mutation rate and the genetic fitness while assuming the allele has no indirect effects) then it implies indirect selection is occurring.

Fisher's fundamental theorem states that the intrinsic rate of increase in fitness (r) of any organism at any time is equal to its genetic variance in fitness at that time (Fisher, 1930). This essentially means that evolution is most rapid when diversity is highest, or alternatively, the fittest allele soon goes into fixation, at which time genetic diversity falls to zero and fitness becomes constant.

The conclusion from a long-running debate was that Fisher's theorem would not be applicable to real life populations (Kimura, 1958; Li, 1967; Karlin and Feldman, 1970). Price (1972) pointed out, however, that this theorem applies only to a partial increase in r caused by natural selection. In other words a mutation will cause a transient change in r, not a long-term change in r.

Witting (2000) negates this view and states that Fisher's r is not a suitable measure of selection in environments characterised by density-dependent competitive interactions.

1.2.3 Frequency-dependent maintenance of genetic diversity

Life-history strategies may be maintained by frequency-dependence. Negative frequency-dependence means the success of a strategy is dependent on the number of other individuals adopting the same strategy, the more that adopt the strategy, the less successful is this strategy.

For example in *Salmo salar* (Atlantic Salmon), smaller males develop whose success depends on there being a sufficient supply of large males to parasitise; likewise in digger wasps, where sufficient nests have to exist for the strategy of parasitism (by 'patrolling' males) to be successful (Frazier, 1997).

Therefore the advantages of a particular strategy become less as the proportion of organisms exhibiting the phenotype that is exploited, become fewer. This means that the success of strategies is dependent on the existence of other strategies and is therefore upholding polymorphic diversity.

1.2.4 Density-dependent maintenance of genetic diversity

In addition to frequency-dependence maintaining diversity it is possible for the expression of different phenotypes to be density-dependent (Sinervo et al., 2000). In such cases, the presumption is that if population densities are fluctuating, the genetic diversity is maintained by density-dependent fitness of the different phenotypes, therefore giving one phenotype better chances of producing viable offspring than the other at different densities. Sinervo et al.

(2000) showed that both density and frequency-dependence can exist at the same time in lizards. Here orange-throated females produced many small eggs and were favoured at low densities where fitness is most strongly related to the population growth rate (MacArthur and Wilson, 1967; Charlesworth, 1994). Conversely yellow-throated females produced large eggs at high density where fitness is most strongly related to competitive ability. Larger individuals with fewer larger eggs implies more competitive individuals (K-selection). The frequency-dependence was shown as being that large eggs had better survival chances, when rare.

Density-dependent morph determination is also shown in the higher melanin concentration of the bodies of many insect species in high-density populations, which give a higher level of protection against pathogens. Pathogen infection is more likely in higher densities (Reeson et al., 1998).

The model organism in this study (see section 1.3), *S. berlesei*, exhibits density-dependent morph determination. In this species, two morphs exist: a fighter male, which develops at low population densities, and a normal male, which develops at greater population densities. Morph determination is primarily thought of to be driven by population density through a chemical cue related to density (Timms et al., 1980).

1.2.5 Local adaptation

Local adaptation can be seen as a reaction to long term spatial heterogeneity, which allows a degree of diversification, but not strong enough for allopatric

speciation. This local adaptation implies that spatial heterogeneity maintains genetic diversity (Galloway and Fenster, 2000; Lively and Dybdahl, 2000; Kraaijeveld and Godfray, 2001).

1.2.6 Limiting and stabilising intra-specific diversity through bet-hedging in fluctuating spatial and temporal environments

The main consideration of bet hedging (Gillespie, 1974, 1977; Cooper and Kaplan, 1982; Sibly et al., 1991) is that organisms have adapted to both the mean of an environmental condition (e.g. wind, temperature), and also its variability. The term bet hedging was introduced by Slatkin (1974). Here the unpredictable nature of an environment results in the evolution of phenotypes, which are adapted to environmental unpredictability (Seger and Brockman, 1987; Phillippi and Seger, 1989; Sibly et al., 1991).

Bet-hedging phenotypes arise from selection reducing the variance in fitness across all possible environmental states, even at a cost to arithmetic mean reproductive success (Gillespie, 1974; Slatkin, 1974; Gillespie, 1977; Phillippi and Seger, 1989). As, the geometric mean (the nth root of the product of n numbers) becomes smaller relative to the arithmetic mean as variance increases, environmental variance favours geometric mean fitness over arithmetic mean fitness (Gillespie, 1974; Slatkin, 1974; Gillespie, 1977; Phillippi and Seger, 1989).

The strategy can be seen to work in vernal pool fairy shrimp. The shrimp

hatch shortly after the pools begin to fill. The shrimp reproduce quickly and the eggs form a protective coating and settle into the soil as cysts. As a season begins there is no obvious sign that the pool will remain wet for a period long enough to allow reproduction. Thus if all the eggs from the previous generation hatch, the result could be local extinction, so only a proportion hatch each year. The potential for extinction is a clear example of how maximising the geometric mean can be seen as superior to maximising the arithmetic mean (Simovich and Hathaway, 1997).

Seger and Brockman (1987) identified two different bet hedging strategies, conservative bet hedging versus (vs) diversified bet hedging. The diversified "bet hedger" assumes randomly the role of two specialist strategies, (i.e. good year specialist and a bad year specialist). By adopting this strategy, it has even a higher mean fitness than a conservative bet hedger, as it assumes (e.g. if the probability for the two specialist strategies is assumed to be 50:50) the average between the two arithmetic fitnesses. This has been shown for desert annuals by Cohen (1993), diapause in arthropods by Istock (1981) and is suspected for offspring size in a theoretical approach by Cooper and Kaplan (1982).

1.3 The andropolymorphic study system

Sancassania berlesei has been recorded since 1882 when Berlese (1882) published a work on polymorphism in some Acari and describes the polymorphic male form of *Analges sp.* Canestrini (1888) showed that a species of *Rhizoglyphus* forms two different types; one whose third pairs of legs is similar to the females and one with the third pair of legs enlarged and ending with a claw. Zakhvatkin (1959) states that the polymorphic males found in the genera *Sancassania* and *Rhizoglyphus* are just examples of many genera of the subfamily Acarina which exhibit andropolymorphism.

1.3.1 General biology

S. berlesei is a mite of about 0.6 - 1 mm adult size. It develops over egg, larvae, protonymph, tritonymph to an adult (Hughes, 1976) (See figure 1.3). The development time from egg to adult of the polyphagous *S. berlesei* was fixed to 159 hours on yeast at 25 °C (Hughes, 1976). Maurya (1982) tested 45 different foods and found that dried yeast proved to be most effective to mass rear *S. berlesei*. Acarid mites whole development cycle (as death, development time, number of offspring) is sensitive to cold temperatures, low atmospheric pressures , low humidity and excess humidity (Zdarkova and Voracek, 1993). Field populations of mites are therefore likely to experience environmentally induced mortality, and so changes in population sizes, in addition to changes in population size caused by changes in resource availability.

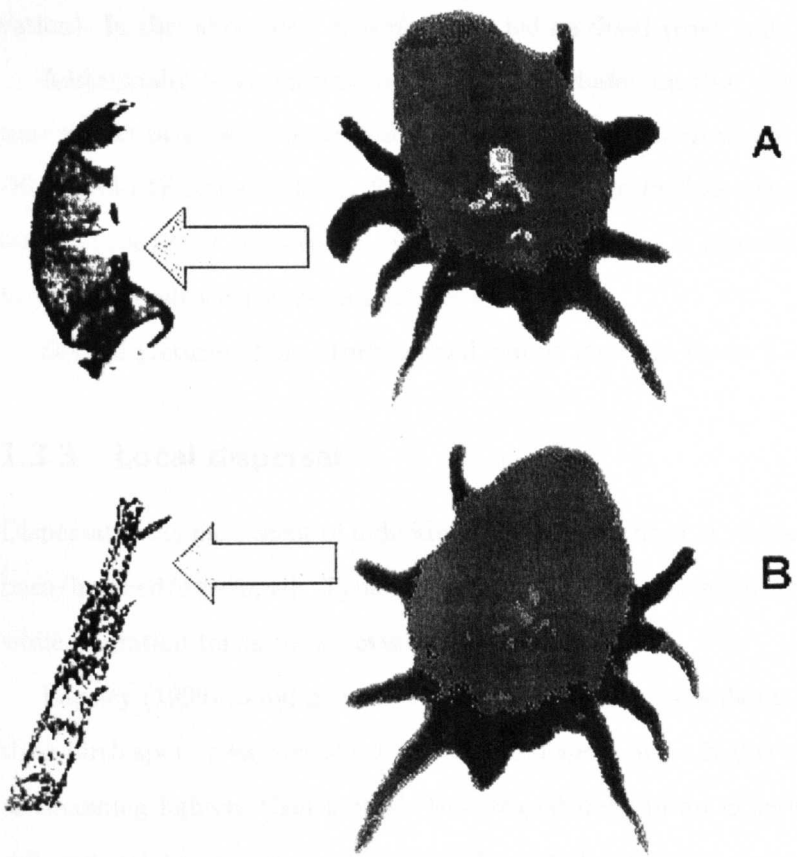

Fig. 1.1: *Males of the species S. berlesei. A) fighter B) non-fighter with corresponding picture of one leg of their third leg pair.*

1.3.2 Diet

S. berlesei generally has a broad diet and is recorded as feeding on dead animals and can even survive, but not grow, on filter paper (personal observation). In the laboratory, *S. berlesei* are fed on dried yeast balls.

Additionally it is phoretic on scarab and chafer beetles, during which time it may take up some nutrients (although the mechanisms are unknown) (Houck and O'Connor, 1991). *S. berlesei* is also described as a species which feeds on root-knot nematodes (Sell, 1988). *S. berlesei* is generally thought to be a generalist consumer and detritivore.

Several pictures of the study animal can be found in figure 1.2.

1.3.3 Local dispersal

Dispersal is the movement of individuals away from the area where they were born/hatched/developed. Typically dispersal is a non-reversible movement, while migration tends to be reversible movement.

Lapsley (1999) found *S. berlesei* dispersing locally by walking away from their birth spot. New arrivals at unpopulated spots had a higher probability of becoming fighters than animals that stayed put. In an experiment with different patches connected by tubes, the tested maximum dispersal length for patches without food was 28 cm. This attracted significantly fewer mites than closer patches (7,14,21 cm). *S. berlesei* dispersed more frequently longer distances for food in an experiment where food was provided in patches further away from the birth spot. This only occurred when food in the

birth patch was low. Otherwise the animals dispersed in significantly lower numbers.

1.3.4 Long distance dispersal

Under adverse conditions a deuteronymph is developed, which does not feed, is more sclerotised and is the animal's long-distance dispersal form (Hughes, 1976; Crocker et al., 1992). *S. berlesei* deuteronymps are phoretic and have been found on 16 species of Scarabidae. Across the 16 species, 0-85% of individuals had attached hypopi, with up to 25.4 mites per beetle on average (and a maximum of 300) (Crocker et al., 1992).

Host size, host sex, year and date in some species influenced the mite densities, but no overall influence over species could be found regarding these factors (Crocker et al., 1992). The relative ranking of beetle species as hosts tended to be consistent from year to year and species which reproduced at the same time in the same habitat, had widely varied levels of infestation (Crocker et al., 1992).

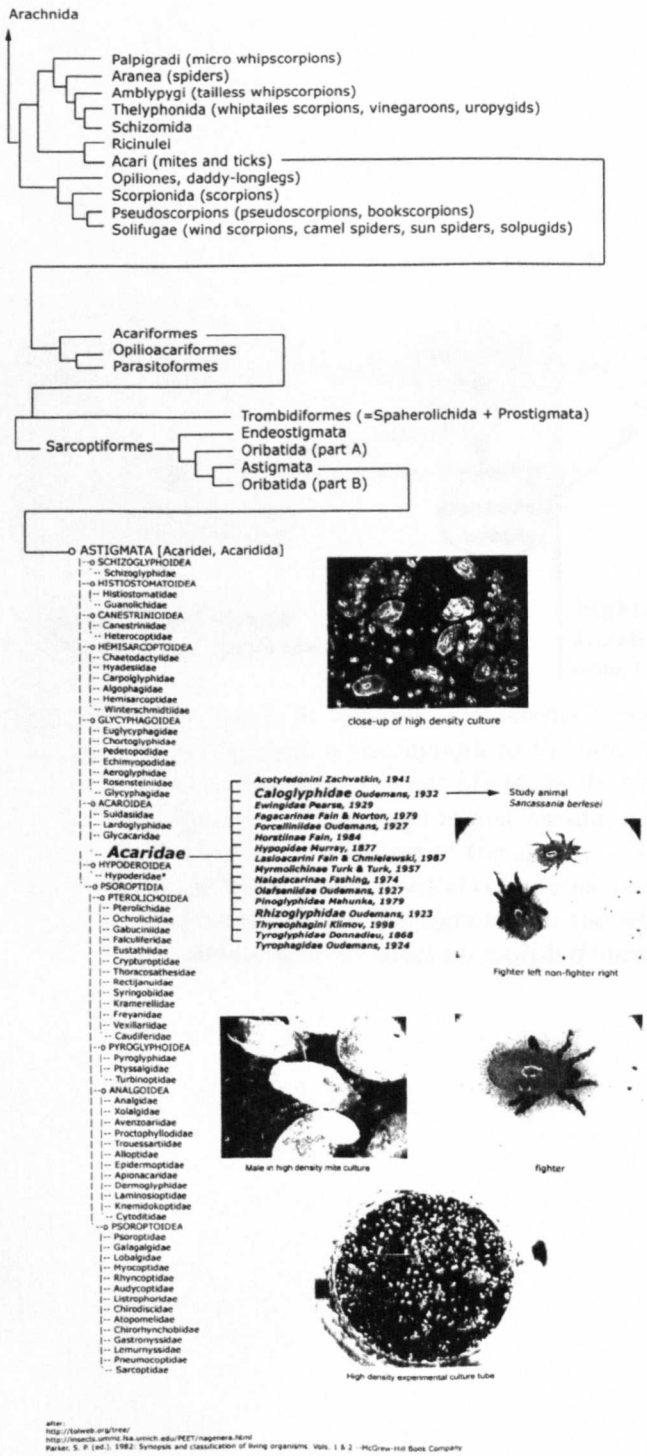

Fig. 1.2: *Classification of S. berlesei within the Arachnida.* *After* http://tolweb.org/tree/; http://insects.ummz.lsa.umich.edu/PEET/nagenera.html; *Parker, S. P. (ed.), 1982: Synopsis and classification of living organisms. Vols. 1 & 2, McGraw Hill Book Company, New York*

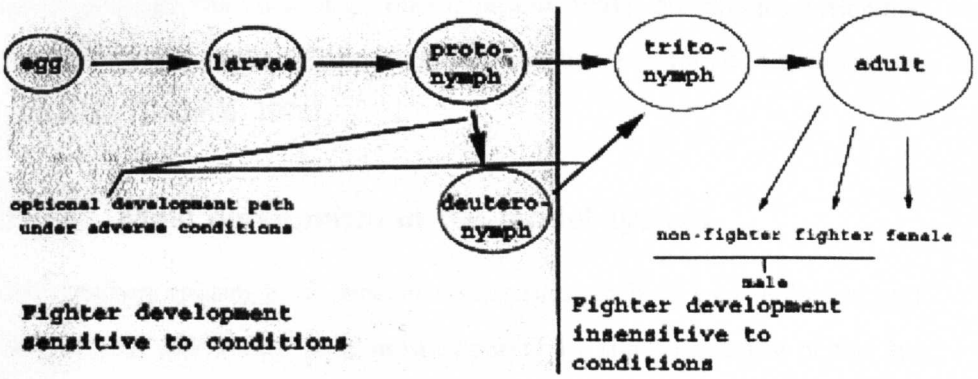

Fig. 1.3: *Life-cycle of S. berlesei. Development goes from egg to larva to a protonymph, a tritonymph to the adult. Under adverse conditions, optionally, another life stage develops, the deuteronymph or hypopus. This stage is non feeding and is the (mostly) long distance dispersal stage of the species. The grey area marks the region where environmental conditions have an effect on the development of fighters/non-fighters. In the white area environmental conditions have no effect on morph determination.*

1.3.5 Mating

Parthenogenesis is absent (Timms et al., 1981a) in *S. berlesei*. Mating occurs continuously from after the last moult. The last male mating with a female fertilises the most eggs (Radwan, 1991), but the proportion decreases as the time between first and second mating increases (Radwan, 1991). Virgin males copulate longer than non-virgin ones (Radwan, 1991). No kin recognition has been found in *S. berlesei* (Woodring, 1969), as has been found in *Sancassania anomalus* (Radwan, 1993).

1.3.6 Male dimorphism in the model system

Andropolymorphism in *S. berlesei* occurs through both morphological and behavioural differences. It is most apparent by the appearance of the 3rd leg pair (Timms et al., 1981a,b) (see figure 1.1). The fighter possesses a thickened and sharply terminated third pair of legs, which are used to puncture the cuticle of other males, while non-fighter morphs have unmodified legs (Radwan, 1995). According to (Woodring, 1969) there are four different types of morphs that occur in Acaridae. His classification describes two basic body shapes. Firstly the homeotype, which has a rounded body and short dorsal setae and the bimotype, with a more elongated body and longer setae. The homeotype is further divided in the homeomorph (non-fighter) and the heteromorph (fighter). Secondly, the bimotype is divided into the bimorph (non-fighter) and the pleomorph (fighter). Woodring (1969) found all these types clearly in *S. anomalus* and found a regular 20% pleomor-

phic/homeomorphic male ratio of *S. anomalus*. The classification of the homeo- vs the bimotype is dubious regarding its biological relevance. It might reflect environmental effects of age and nutrition and cannot easily be identified in the laboratory (personal observation). For the rest of this work only the fighter vs non-fighter polymorphism is considered, as it is readily identifiable and distinguishable.

There is strong density-dependence in growth; as a result of this animals reared at low density tend to be larger (Beckerman et al., 2002, 2003). Therefore males of *S. berlesei* are larger if they are isolated earlier from stock cultures (personal observation). Fighter males isolated at the protonymph stage grew larger than non-fighter males isolated at the tritonymph state. There was no difference in the size of the two male morphs, if they were isolated as larvae and raised together in small groups (Radwan, 1992) due to higher per capita food. This is probably a result of competition for food, but has not yet been confirmed.

In *S. berlesei* it is common to find males which are intermediate between fighters and non-fighters: they have one enlarged 3rd leg with the other having the normal build (Timms et al., 1981a,b). This morph is called ambiomorph by Nesbitt (1993) and intermorph by Timms et al. (1981a). Nesbitt (1993) suggests that ambiomorphism might be associated with the first division of the zygote into the daughter cells. Since the two sides of the body of this form are mirror images of each other, except for the hind legs and the difference in the length and robustness of some of the dorsal setae, this would be consistent with a non-disjunction of the chromosome pair that

carry the genes responsible for the heteromorphic type of male. Nevertheless this explanation is purely theoretical. Ambiomorphs cannot be reliably reproduced in the laboratory (personal observation).

Woodring (1969) found the highest rate of fighters at 20° temperature and this rate decreased below and over this temperature. At any given temperature the rate is highest when the animals were fed on animal tissue, lowest when fed on yeast. This suggests that morph determination depends not only on density; this is a subject covered in detail later in this thesis.

1.4 Assessing the outcome of evolution

To predict the outcome of evolution in *S. berlesei* it is necessary to have a performance measure to compare different life-history strategies. This value is usually termed fitness, but what is fitness exactly?

The measures of fitness used fall approximately into four groups [after (Benton and Grant, 2000)]:

1. based on measures of population growth (such as r)
2. based on measures of reproductive success (such as R_0, LRS (life-time reproductive success) inclusive fitness)
3. based on population size (such as K)
4. others (such as time to extinction)

Here the most commonly used are one to three (Benton and Grant, 2000). Brommer (2000) recently reviewed the history of fitness measures.

1.4.1 Non-dynamic fitness estimates

The use of r assumes that the population size is unbounded and the environment is constant (Charlesworth, 1994), although Tuljapurkar (1990) derived a stochastic analogue of r termed a, which allows the relaxation of the constant environment assumption. The use of r as fitness implies a constant environment, which either has no density-dependence or the density is not varying over time.

Many populations are in some form of equilibrium where the growth rate approximates zero. This will give us no indication of the fitness of the animals, as the population growth rate, and therefore fitness is constrained to be zero. The same seems to be relevant for measures based on reproductive success, like LRS. In an equilibrium population the average LRS would be one as all animals dying could be replaced by one animal. The apparent paradox that evolution maximises a value that is constrained is illusory. In a population there will be variation between individuals, as the mean LRS of the population would be one, but each individual could have an individual LRS of greater or smaller than one. Therefore LRS cannot be used as fitness measure on population level, but might be valuable on an individual-based level.

The population size at equilibrium, the carrying capacity for the trait in question or K can also be used as a measure of fitness (Brommer, 2000). The fittest strategy for an equilibrium level population is the strategy which transforms limited resources into the most individuals (MacArthur and Wil-

son, 1967; Charlesworth, 1994).

Time to extinction measures an organism's fitness by measuring the time to extinction of one organism, with one trait, against the time to extinction of another organism, with another trait. The fitter organism is that which is assumed to have a longer time to extinction.

Therefore many of these fitness measures require some form of assumption and are practical for different kinds of biological questions (Stearns, 1992). However, many of these assumptions are unrealistic in most cases (Benton and Grant, 2000), as there are few cases where organisms can expect to live in constant environments over a long period of time, or live in density-independent environments.

If a mutant grows into a population at equilibrium, it might reach, after taking over from the previous genotype, also an equilibrium. Being then the so-called resident, the former mutant might then become increasingly rare, when invaded by new mutants which are growing into the population. Non-dynamic fitness estimates, while being able to track short time fitness, do not take into account that with invading (becoming the resident genotype) they have actively changed the dynamics and might be susceptible to invasion themselves (Diekmann et al., 1999).

1.4.2 Dynamic fitness estimates

Evolution proceeds by successive invasions of mutant strategies into populations of residents. The outcome of evolution is properly, therefore, predicted

by analysis based on invasion of mutants into resident populations (Metz et al., 1992; Rand et al., 1994; Ferriere and Gato, 1995; Mylius and Diekmann, 1995; Gurney and Middleton, 1996; Benton and Grant, 1999b).

ESS

Invasion analysis is based on a concept called Evolutionarily Stable Strategy (ESS) which is according to Maynard Smith and Price (1973):

> *"A strategy such that if all members of the population adopt it, then no mutation can invade the population under the influence of selection"*

ESS theory, a branch of game theory, is based on the idea of "evolutionary games", where different strategies "play" over evolutionary time scales, with gene frequencies evolving towards a stable state (Maynard Smith and Price, 1973).

As an example, the "Hawk-Dove" game uses the analogy of a "Dove" strategy vs a "Hawk" strategy. The "Dove" (being the "gentle" strategy) retreats if the opponent escalates the contest, whereas the "Hawk" always escalates the contest and continues until injured or until the opponent retreats. If all individuals are using a "Dove" strategy, this strategy is more likely to increase individual fitness. However, invading Hawk strategists always defeat individuals adopting the "Dove" strategy.

So organisms do not necessarily have to optimise their strategy, it is sufficient to be better than your competitors.

Estimating ESS using a Lyapunov exponent ϑ

Invasion is assessed by estimating the rate of spread (= population growth rate) of a mutant strategy when rare. This quantity is technically the dominant Lyapunov exponent (Metz et al., 1992), termed invasion exponent ϑ (Rand et al., 1994). Under constant conditions, ϑ is equivalent to $\lambda = e^r$ (the population rate of increase) and under constant density-dependent conditions ϑ equals the LRS. Under stochastic or stochastic density-dependent conditions ϑ has to be estimated explicitly; either analytical or by numerical methods. The outcome of evolution will be the strategy that can invade all others, but is not itself invadable.

Adaptive dynamics

Adaptive Dynamics is the study of adaptation whilst simultaneously considering the organisms environment, which may include population dynamics.

Adaptive dynamics is based around modelling the invasion of mutant strategies into populations of residents. It is an extension of ESS analysis, as it looks for the end points of evolution via successive invasions. A common method of displaying the analytical results is via Pairwise Invasibility Plots (PIP), which show when a resident can be invaded, and the invasion functions give insight into the stability of the singular points (Diekmann, 1996) (see figure 1.4).

The process when a monomorphic population becomes dimorphic, through continuous differentiation of the initially similar strategies, is called evolu-

tionary branching. The singular point (see figure 1.4) at which evolutionary branching starts is called a branching point (Metz et al., 1996). A prerequisite for evolutionary branching is that directional selection drives the population towards a fitness minimum. In sexual populations with random mating, the continual production of intermediate phenotypes from two branches prevents evolutionary branching. In contrast, when mating is assortative for the ecological characters under study, evolutionary branching is possible in sexual populations and can lead to speciation. Doebeli and Diekmann (2000) conclude that evolutionary branching offers a general basis for understanding adaptive speciation and radiation under a wide range of different ecological conditions.

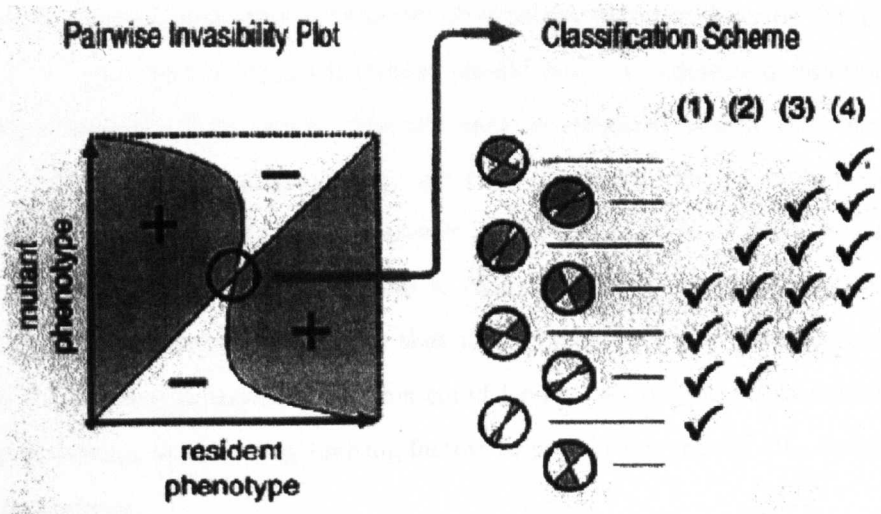

Fig. 1.4: *Pairwise Invasibility Plot and the classification of evolutionary singular points. The adaptive dynamics invasion function of a particular ecological system defines a Pairwise Invasibility Plot for resident and mutant phenotypes. When the invasion function is positive for a particular pair of phenotypes, the resident may be replaced by the invading mutant. Intersections of the invasion function are zero contour line at these singular points suffice to answer four separate questions: (1) Is a singular phenotype immune to invasions by neighbouring phenotypes? (2) When starting from neighbouring phenotypes, do successful invaders lie closer to the singular one? (3) Is the singular phenotype capable of invading into all its neighbouring types? (4) When considering a pair of neighbouring phenotypes to both sides of a singular one, can they invade into each other? From (Diekmann, 1996)*

1.5 Tools

Mathematical and computational models are used to represent mathematically the biological questions under observation. Models describe complex phenomena, and using mathematics, should help to understand functional relationships within nature, like the ones investigated within this thesis. Models can help to discover laws and rules of nature. For example a simple model like the exponential growth model ($N_{t+1} = N_t e^r t$ with t=time; N_t=population abundance at time t; N_{t+1}=population abundance at time $t + 1$; r=growth rate) can show that if a population would be unbounded it would grow exponentially. This could lead to a) looking for unbounded populations, or b) finding limiting factors to population growth, like density-dependence.

Incorporating density-dependence, for example, in the model above, may show a more realistic picture of population dynamics. This can be compared to real life populations and, if a model describes the dynamics of a population under investigation, may allow the possibility of predicting population dynamics.

Stage structured models, like the LPA (Larvae, Pupae, Adults) model of *Tribolium*, can describe an arthropod system as used in this study (Constantino et al., 1995; Dennis et al., 2001). Stage structured models, like matrix models (Caswell, 2000), can split the population into several different stages. Matrix models operate on predefined stages of a population, being this age or size or instar. Nevertheless they do not operate in continuous

but in discrete time. A discrete time matrix model was used in this study to make predictions about the possible influence of stochasticity on life-history evolution, concentrating on the several discrete life stages of the study system under question, which where grouped into egg, juveniles and adults. Modelling in discrete time steps was considered to be sufficient detail, as the data collected to "prime" this study's matrix model could only be taken realistically is discrete time-steps (days).

To assess the possible outcomes of evolution, invasion based modelling was used in this study, to be able to predict evolution in a density-dependent and stochastic environment. Density-dependence was included in the model after analysis of laboratory data showed that several life-history traits were density-dependent, although this density dependence was not always linear (straightforward) on a logarithmic scale. To reflect the animals in the laboratory cultures, an explicit spatial component was not modelled at this stage, and was only included on a small scale in an individual-based model reflecting the animal movements as a particle system.

Individual-based configuration models work on an individual basis. They have their basis in differing individuals, whose diversity influence their surroundings (Batschelder and Williams, 1995; Ferreira, 1995; Axelesen et al., 1997; Carnahan et al., 1997; Beecham and Farnsworth, 1998). Individual-based distribution models basically build on individual-based configuration models, but filter the individual into sub-classes based on certain traits (e.g. age, height, build) and look at the behaviour and interaction of these sub-classes (DeAngelis and Rose, 1992). Individual-based models, are widely used

in population dynamics modelling (Batschelder and Williams, 1995; Ferreira, 1995; Axelesen et al., 1997; Carnahan et al., 1997; Beecham and Farnsworth, 1998).

Given the fighter morph determination where fighters occur in low numbers, it seemed to be essential to investigate possible effects of one or a few individuals on a starting population. An individual-based model, defined by its very name, seemed to be the most suitable for this purpose. Additionally, fighting requires spatial interaction. This resulted in a choice of an spatial individual-based model to investigate the effect of eliminating competitors from a starting population, where low density or low animal numbers are common and have a higher influence on future generations. Few fighters in a starting population should have an advantage, as they father many offspring. This should be highly beneficiary for a fighter's genes, as they are the ancestors of all future populations.

1.6 The study

In this study, a detailed description of the entire life-history will be incorporated into evolutionary models that include both density-dependence and environmental variability. It will therefore be possible to investigate the mechanisms of cost and benefits that lead to the two coexisting strategies.

As fighters of *S. berlesei* are commonly found in cultures with low densities, previous studies have suggested that there is a trade-off between ability to monopolise females and time spent fighting, such that at higher densities

fighters get a smaller share of paternity than non-fighters. If the population has to vary, as seems likely for the fighter morph to be maintained, (such that the population density is sometimes sufficiently low to make fighting a profitable strategy), the key question addressed is under what conditions of variability will fighting be maintained by selection and, if so, what is the threshold density below which fighters should develop?

To study the influence of population dynamics on polymorphism in *S. berlesei*, a density-dependent stochastic matrix model will be used to impose variation on the population dynamics. Using invasion analysis, the evolutionarily stable strategy (ESS) will be determined by linking the probability of becoming a fighter to population density.

Using an individual-based matrix model, the possibility that the fighter morph is determined by frequency rather than by density is investigated. By investigating frequency-dependent and density-dependent mechanisms, it will be possible to generalise across different species of mites. In the family of the study organism several species exhibit a similar polymorphism as the model organism, but with a different kind of morph determination.

The conclusion will amalgamate the results of the previous chapters and present a possible scenario for the evolutionary constraints of the different kinds of morph determination.

2. INCIDENCE, COSTS AND BENEFITS OF BEING A FIGHTER

Abstract

1.) Fighters primarily develop at low population densities;
2.) Though the proportion of males becoming fighters at any given density may change over time.
3.) This change is likely to be due to condition-dependence.
4.) Data is presented to illuminate these matters and a model is developed linking fighter development to the costs of being a fighter (in terms of survival) and
5.) the benefits of being a fighter (in terms of fecundity).

2.1 Introduction

S. berlesei's morph determination shows an environmental influence as found by Timms et al. (1980), Radwan (1995) and Ballard (1997). At low population density fighters develop, and they cannot be found at higher population densities (Radwan, 1995). Ballard (1997) suggested that with increasing density the fighter finds less time for mate interactions but is overwhelmed by fight interactions indicating a density-dependence.

One would define density here as number/area (animals move on flat ground mostly in two dimensions) and to a small percentage also as num-

ber/volume (as occasionally animals move over each other). The influence of density is likely important, as animals have to interact (mate, fight) and a larger area results in fewer interactions, as the animals meet fewer times. A fighter has to fight and kill (therefore meet) another male to "cash-in" on his advantage to be able to kill other males. Lapsley (1999) found fighters in a large coupled arena, although the numbers of mites exceeded the numbers where fighters would usually be found. The animals have to be in close proximity (or densely packed) to react in the way described in this chapter. So I interpret this as a density-dependent effect, rather than a population size effect. As experiments reported in this study use the same size vials, population density is directly proportional to population size.

The development of fighters at low densities is environmentally conditioned. Timms et al. (1980) investigated the cues and found that a chemical found in large colonies acted as a suppressor of fighter development. Timms et al. (1980) also researched whether food texture or the ether extract taken from a mite population had an influence on fighter development. In both cases no influence on fighter development was found.

If fighters only develop at low densities, one must ask why this may have evolved. For it to be adaptive, the costs must be lower than the benefits at low densities and vice versa at high densities. Costs and benefits are both levied in fitness terms, so fighters should expect to achieve a higher reproductive success than non-fighters at low densities, with the reverse at high densities. A fighter in a low density situation only has to kill its (few) competitors and can then subsequently monopolise the females. This is facilitated by

fighters developing faster than non-fighters (Radwan, 1995). Conversely at high densities, fighters should have a lower reproductive success, perhaps because they spend more time fighting than mating and competitors are too common to enable them to monopolise females. One therefore expects a relationship between population density and fighter fitness, as suggested by (Radwan, 1993).

Timms et al. (1980) found significant differences between fighters and non-fighters. The investigation established that populations of fighter males of *S. berlesei* live longer than non-fighter males, and fighter males produce more young earlier than non-fighter males, although there is no difference in egg numbers laid by females of each type.

Woodring (1969) and Nesbitt (1993) discovered small subtle polymorphisms. Fighters and non-fighters may vary (apparently discontinuously) in size and shape, though Nesbitt (1993) found no difference in their ability to feed, mate and move in many morphs of various *Sancassania* species.

Normally fighters only develop if they are reared from pre-tritonymph individuals. The normal procedure to obtain fighters is to pick pre-tritonymph larvae from a stock culture and allow them to develop in isolation or at low densities.

In previous studies it was assumed that the time or stage to the tritonymph state had no influence on the percentage of fighters developing at certain densities, and that only two discrete states existed. Individuals may "switch" onto the fighter developmental pathway if they experience the necessary environment early in development, but after the tritonymph stage, regardless of

the environment, fighter development is not possible. A relationship between stage or time and morph determination was assumed to be non existent.

2.1.1 Investigating the occurrence, costs and benefits of the fighter morph

In this chapter an investigation of the incidence, costs and benefits of developing into a fighter will be made, lower densities were investigated in more detail and it is demonstrated with a model of reproductive success, why it might be beneficial for a mite of the species *S. berlesei* to become a fighter not only when it is possible to totally monopolise its opposition (as in densities one or two pairs), but also when the densities are a bit higher or if the mite faces fighting opposition.

Therefore the following questions will be asked:

1. Is there a threshold density above which fighters do not develop any more, or is the relationship continuous?
2. Is density the only cue for fighter morph determination?
3. Does the switch in the developmental path of the mite occur at a discrete point in time?
4. Can we measure the benefits of being a fighter in terms of fecundity;
5. and the costs in terms of survival?

Density-dependent morph determination

At low population density, fighters develop and cannot be found in higher population densities (Radwan, 1995). This relationship could be continuous or discontinuous. If discontinuous, there could be a threshold density below which all males become fighters. If continuous the probability of becoming a fighter could vary smoothly as density changes, with no 'stepwise' threshold.

Condition-dependent morph determination

As well as the density being a determinant of the probability of becoming a fighter, Radwan (1995) found an influence of environment, notably food availability. Poorly fed males were less likely to become fighters.

Fighter fecundity in all environmental conditions

In order to assess the costs and benefits of developing into a fighter, information on the fecundity of the different morphs under different conditions is required. First, the fecundity of the two morphs with females which are either virgin or non-virgin (but sperm depleted) is assessed. *S. berlesei* disperses as hypopi (a non-feeding additional life-stage, that develops in adverse conditions, and is *S. berlesei*'s dispersal stage) on beetles, so fighters developed at low densities in a newly colonised patch are likely to encounter virgin females. Fighters, when they develop at high densities, would be more likely to encounter non-virgin females. On the other hand females at a new patch at low densities will be more likely to encounter fighters and at high den-

sity more likely non-fighters. At high densities per capita food availability is reduced. Females mating with fighters and non-fighters might encounter animals with different pre-tritonymph life-history. As the individual life-history up to deuteronymph stage may occur on a different patch from where the animals actually mate, it is possible, that animals with a low food life-history (up to dispersal stage) arrive at a high food site, if emigrating from previous even worse conditions. Although hypopi arise in adverse conditions (Ballard, 1997), this does not mean that they experienced bad conditions before they became hypopi. This means hypopi could have grown up as eggs and larvae with sufficient conditions, and somewhere in their protonymph stage bad conditions arose, which would have turned them into hypopi. Therefore a variety of different feeding conditions can arise at new patches.

Survival probabilities of fighters and non-fighters

If a fighter is in an environment solely with non-fighters, he can kill the non-fighters and will himself encounter no hostilities. But if a fighter is together with other fighters he will obviously encounter fighters himself. As fighters actively seek out other males and initiate fights (personal observation) one would expect that the probability of survival of a fighter with other fighters present sinks when compared to a non-fighter, as non-fighters try to evade fights if engaged in them.

2.2 Methods

Data analysis was conducted in S-Plus 2000, besides the GLMM (General[ised] Linear Mixed Model) analyses, which was calculated in Genstat using the iterative reweighted restricted maximum likelihood method (IR-REML). GLMMs are generalised linear models with normally distributed random effects in the linear predictor. Non-linear regression models were tested and compared with residual least squares. General (or generalised) linear models (GLM) were used were appropriate and further details about the form of GLM employed are given when the test results are given. The residuals were checked for non-violation of normality of the residuals vs the fitted values and heteroscedacity.

Percentage of fighters developing from isolated individuals at egg stage

To establish any time or stage dependence in the morph determination, 100 eggs and 100 larvae were picked from a 1996 stock culture and placed in 20 mm wide and 50 mm high plastic vials. The vials were lined with Plaster of Paris ($CaSO_4 \cdot {}^1/_2\ H_2O$) which was dampened to maintain humidity. Food was supplied in the form of a small ball of yeast.

The tubes were placed in a 24 °C incubator and left for seven days. After this time the number of males (fighters and non-fighters) and females were counted.

Density-dependent morph determination

An experiment was conducted in 1998 with the standard conditions and equipment described earlier. Two hundred and ten vials were set up with larvae isolated at pre-tritonymph stage and fed one ball of yeast per day. The density of animals in these tubes was 1, 2, 5, 10. After a week the number of developed fighters were noted.

Condition-dependent morph determination

An experiment was conducted to consider the number of fighters developing at different levels of food, differing times and amount of food supplied at varying densities. Food was supplied in two different regimes, low and high food, whereby low food consisted of a grain of yeast per day and high food of five balls of yeast per day.

The food was given either from the beginning of the experiment, or after a five day delay. Pre-tritonymph larvae were selected from tubes, which had been taken from the stock cultures. These larvae were then separated into the experimental tubes. The number of fighters, non-fighters and females was recorded. The total number of tubes was 40.

Fighter fecundity in a good quality, resource stable environment

To estimate the fecundities and survival probabilities of fighters and non-fighters an experiment was conducted in which fighters were brought together at differing densities with non-fighters and females. The number of animals

and their phenotype was recorded. During the day of the experiment at hourly intervals the number of matings and fights between fighters and non-fighters was noted. The animals were put together at the densities 5, 10, 20 and 50. Fighters, non-fighters and females were added according to table 2.1. Detailed information was taken on densities 5 and 10 (see section 2.1.1), where the advantages for fighters should slowly tail. To get a strong signal on high extreme combinations detailed information was collected at densities 20 and 50, where the advantage of being a fighter should not be non-existent. Fighters and their reactions in densities <5 were frequently observed in other experiments, so were not tested here due to time restrictions.

$\delta =$	5	10	20	50
	$1_f,4_{nf},5_{fe}\cdot 2$	$1_f,9_{nf},10_{fe}$	-	$1_f,49_{nf},50_{fe}$
	$2_f,3_{nf},5_{fe}\cdot 2$	$2_f,8_{nf},10_{fe}$	-	-
	$3_f,2_{nf},5_{fe}\cdot 2$	-	-	-
	$4_f,1_{nf},5_{fe}\cdot 2$	-	-	-
	$5_f,0_{nf},5_{fe}\cdot 2$	$5_f,5_{nf},10_{fe}$	-	-
	-	$7_f,3_{nf},10_{fe}$	-	-
	-	$10_f,0_{nf},10_{fe}$	-	-
	-	-	$20_f,0_{nf},20_{fe}$	-
	-	-	-	$50_f,0_{nf},50_{fe}$

Tab. 2.1: *Experimental setup: δ=density; f = fighter; nf = non-fighter; fe = female; x_T = x animal(s) of type T. $\cdot 2$ = two repetitions. Experiment unbalanced due to time/laboratory restrictions. To gain equal sized fighters, non-fighters and females and to account for deaths over 400 tubes had to be reared simultaneously, which was the maximum possible to handle. Therefore point measurements were taken.*

Fighter fecundity in all environmental conditions

Animals were taken as larvae from a culture of about 1000 animals which was itself taken from the stock cultures, to match experimental conditions of a previous experiment which looked at general life-history parameters, (described in the next chapter). Non-virgin females were produced by rearing them as larvae and by then bringing them together for one day with about ten males, fighters and non-fighters in equal proportions. After one day the females were isolated, until no more eggs had been laid. Rearing larvae, in isolation produced virgin females. Rearing larvae in isolation produced non-fighters and fighters. Sixty four animals were reared using a crossed experiment using the factors described in table 2.5. Animals were raised on the designated pattern and where kept on the designed feeding regime (high and low food). After the animals had been reared the males and females were paired, resulting in a total amount of 32 tubes. The eggs laid by the females were counted after five days, this being the peak of female productivity under optimal conditions (see chapter 3).

Survival probabilities of fighters and non-fighters

To establish if the survival probabilities of fighters at different levels of densities and fighter densities the surviving animals were recorded in the experiment described in section 2.2 and with the experimental setup of table 2.1. survival probabilities were recorded (see section 2.2).

2.3 Results

Percentage of fighters developing from isolated individuals at egg stage

Out of 100 animals isolated at egg stage 93 developed into adults. Out of these 93 adults, 52 developed into males. Out of these 52 males, 39 developed into fighters. From 100 animals isolated as larvae at pre-tritonymph stage 96 developed into adults. Forty-seven of these 96 adults became males. 37 of these males were fighters. Therefore 75% of males developed into fighters when individuals where isolated at egg stage compared to 79 % when isolated at pre-tritonymph post egg stage. This difference in percentages is not significant ($\chi^2 = 0.025, df = 1, p = 1$).

One can therefore have some confidence that the development switch, when it is possible to become a fighter given the necessary environmental indications, occurs at a discrete time, namely within the tritonymph stage. It is therefore not important at which stage the animal is isolated, given it is before the tritonymph stage.

Density-dependent morph determination

The results with number of vials, mean and SE are given in table 2.2.

density	N	$\bar{x}(P_f)$	$SE(P_f)$
1	59	0.72	0.053
2	59	0.27	0.034
5	20	0.14	0.035
10	70	0.02	0.005
$\sum N$	208		

Tab. 2.2: *Mean percentage $\bar{x}(P_f)$ and standard error $SE(P_f)$ of the probability of developing into a fighter for S. berlesei isolated at pretritonymph stage at density 1 2 5 10 in year 1998.*

A non-linear regression was performed and a relationship between fighter morph determination and density in high food conditions was found ($RSS = 16.9, SE = 0.032, t = 20.96$).

The relationship is represented in equation 2.1 where δ is density and P_f is the probability of developing into a fighter.

$$P_f = \frac{0.69}{\delta} \tag{2.1}$$

Equation 2.1 is graphically demonstrated in figure 2.1.

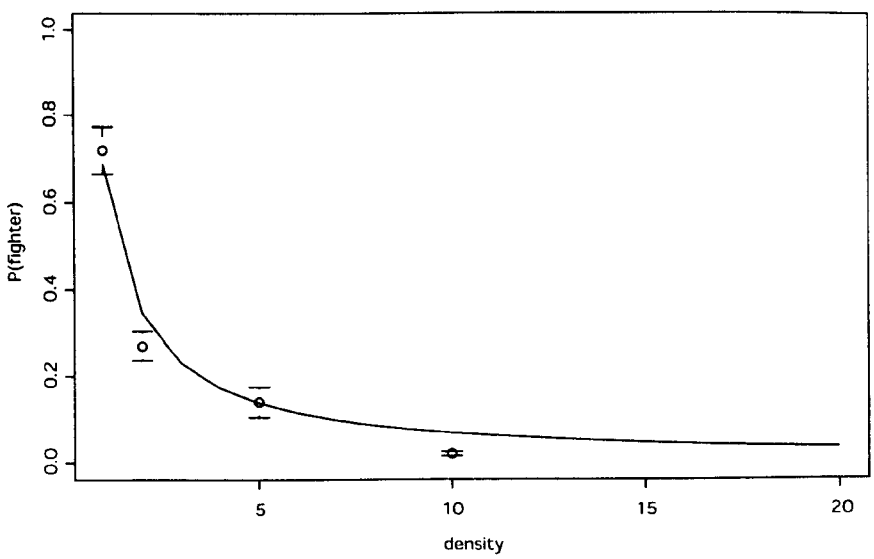

Fig. 2.1: *Relationship as calculated by a non-linear regression between the probability of developing into a fighter [P(fighter)] vs culture density and data points with standard error bars.*

Condition-dependent morph determination

δ	N	high food $Mean \pm SE$	N	low food $Mean \pm SE$
1	3	$0.667 \pm NA$	5	$0.400 \pm NA$
2	2	0.500 ± 0.500	3	0.333 ± 0.167
3	1	$0.000 \pm NA$	3	0.444 ± 0.111
4	2	0.125 ± 0.125		
5	2	0.200 ± 0.000	1	$0.000 \pm NA$
6			2	0.250 ± 0.083
7	2	0.143 ± 0.000		
9			1	$0.111 \pm NA$
10	1	$0.200 \pm NA$		
12			1	$0.167 \pm NA$
13			1	$0.154 \pm NA$
14	2	0.250 ± 0.03		
17			1	$0.175 \pm NA$
21	1	$0.240 \pm NA$		
$\sum N$	16		18	

Tab. 2.3: *Number, mean and SE of the probability of becoming a fighter at two different levels of food at different densities δ. NA=Non available.*

The results are summarised in table 2.3. Six tubes did not complete the experiment, because of premature death of the animals. An analysis of deviance table of a generalised linear model with Poisson error and identity link function (table 2.4) shows a significant effect of food, density and the interaction of density with food. Males in high densities with low food supply had a lower probability of becoming a fighter than with high food provisioning.

	df	$p(Chi)$
$food$	33	0.023
δ	32	0.046
$food:\delta$	31	0.0015

Tab. 2.4: *Analysis of deviance table of generalised linear model with Poisson error and identity link function on number of fighters developed on different levels of food and densities δ.*

Fighter fecundity in all environmental conditions

Results are presented in table 2.5. Animals supplied with food at a low rate and animals experiencing a five-day window without food after birth had a higher mortality. A total of eight vials did not complete the experiment (six tubes with non-fighters and two with fighters). This is not significant ($\chi^2 = 2.16, df = 1, p = 0.106$). Nevertheless a vial in each category completed the test.

A Gaussian GLM was performed and the residuals checked for non-violation of normality of the residuals and heteroscedacity. The model:

$$N_{eggs} \propto Type\ of\ male$$

was the minimum adequate model.

Females fertilised by fighters, pooled over all conditions, laid more eggs ($\bar{x} \approx 80.56$) than non-fighters ($\bar{x} \approx 65$) (Gaussian GLM, n=32, $p < 0.02$).

		fed at birth			fed after 5 days after birth	
		NF	F		NF	F
	V	$52 \pm NA$	64 ± 19.79	V	$0 \pm NA$	81 ± 33.94
low food		n=1	n=2		n=1	n=2
	NV	$91 \pm NA$	55 ± 21.21	NV	$101 \pm NA$	56 ± 79.19
		n=1	n=2		n=1	n=2
	V	32 ± 45.25	124 ± 4.24	V	150 ± 14.14	125 ± 21.92
high food		n=2	n=2		n=2	n=2
	NV	$34 \pm NA$	105 ± 115.96	NV	$60 \pm NA$	34.5 ± 40.30
		n=1	n=2		n=1	n=2

Tab. 2.5: *Mean ± standard deviation and number of vials (n) for number of eggs laid by virgin (V) and non-virgin (NV) females on day of first egg-laying, fed on high and low food which was provided at start or after five days and mated with fighters (F) and non-fighters (NF). NA= Non applicable.*

Fighter fecundity in a good quality, resource stable environment

As *S. berlesei* exhibits sperm competition (Radwan, 1991) the order of matings is relevant. In an experiment comparing reproductive success of males mating first or second with a female, Radwan (1991) estimates that 86% of eggs are fertilised by the second and last mating. However this percentage dropped to 56% when the last mating was more than six hours after the previous mating.

Mate guarding does not exist in *S. berlesei* (Radwan, 1991). Therefore, it seems that one option for *S. berlesei* to eliminate sperm competition would be to kill other fighters and non-fighters as fast as possible and postpone mating for not more than six hours. This pattern could not be recognised in the laboratory where after introduction of fighters and non-fighters into a

test tube, both attempt to start mating nearly instantaneously. In fact the number of matings obtained by fighters is much greater than that of non-fighters. The non-fighters achieve very few matings with fighters present, except in tubes where they significantly outnumber the fighter(s) (corrected for initial fighter/non-fighter numbers and eliminating trials with no non-fighters in them) (one-sided Wilcoxon rank-sum test, n=13, m=18, $p < 0.05$) (see table 2.6).

	Median	n	NA's
fighters	0.37	18	0
non − fighters	0.001	13	5

Tab. 2.6: *Median of hourly per capita mating success obtained by fighters and non-fighters over all densities as calculated by animal numbers by female fecundity and divided by hours. NA's= not available; no non-fighters in the vial initially.*

For simplicity and in order to trace the effect of density-dependence on survival and fighter reproductive success, it is assumed in the following model, that the animals present after one week obtain most of the reproductive success (as the last mating gains most of the fertilisations), and that, whilst both phenotypes are alive, they achieve the same amount of matings (and order of matings). There was no difference in the fecundity of females fertilised by fighters and non-fighters, when the feeding regime (high food) was the same and animals with both feeding times were pooled (two-sided Wilcoxon rank-sum test, n=8, m=6, $p >> 0.05$, see table 2.5). Therefore, when three fighters and two non-fighters are in a population, fighters should gain 60% of all matings and therefore the reproductive success of the non-fighters would

be 40%. After one week the cuticle of the males is sufficiently hardened so that fighting does not result in any further deaths (Timms et al., 1980), so that changes in the proportion of fighters and non-fighters in the starting cohort are unlikely due to fighting after this time period.

	Value	*SE*	$t - value$
c	8.23	0.714	11.52
θ	26.14	1.43	18.25

Tab. 2.7: *Result of non linear regression for female fecundity per day per density pooled over all feeding regimes (see section 3.3.3 and table 3.1), with value, SE and t-value and residual standard error of 18.55 on 961 df.*

The relationship between fecundity (mean egg female^{-1} day^{-1}) and density was fitted with a non linear regression (see table 2.7 and equation 2.2), where c = 8.23, θ=26.14, δ=pair density, y=number of eggs females laid by female per day pooled over all feeding regimes, to get the effect of density-dependence in all conditions.

$$y = c + \frac{\theta}{\delta} \tag{2.2}$$

Although females are able to lay in excess of 80 eggs per day if reared in isolation and with excess food, the data (see section 3.3.3 and table 3.1) was collected from mites reared on high and low food sources and the fit therefore represents the mean of eggs produced over all nutritional conditions on all days.

The proportion of fighters, Φ_F in a total population is:

$$\Phi_F = \frac{N_F}{N_{NF} + N_F} \tag{2.3}$$

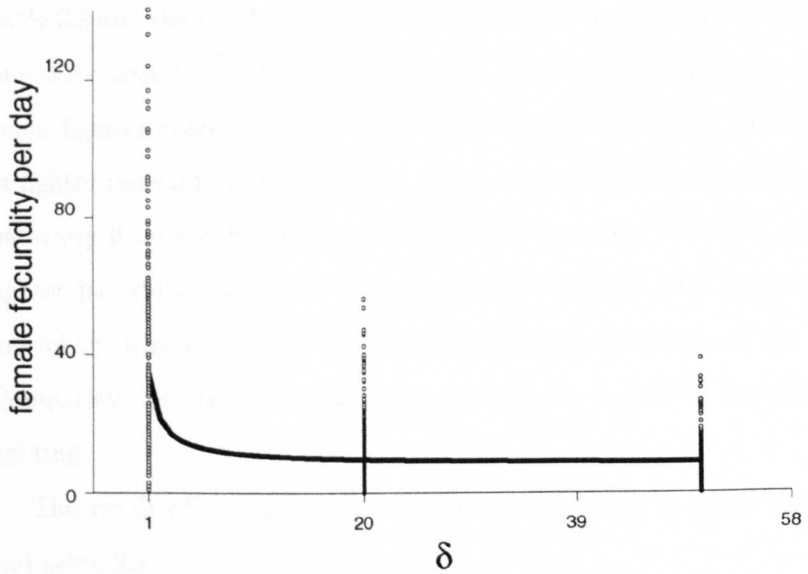

Fig. 2.2: *Non linear regression and data (see section 3.3.3 and table 3.1) for female fecundity per day pooled over all feeding regimes vs δ=density in pairs.*

where N_F is the number of fighters and N_{NF} is the number of non-fighters.

From this one can estimate the number of eggs a fighter can achieve in competition with non-fighters:

$$N_{F,eggs} = \frac{(\Phi_F) \cdot (c + \frac{\theta}{N_{Fe}})}{N_F} \tag{2.4}$$

where, $N_{F,eggs}$ = fighter paternity, N_F = number of fighters, N_{NF} = number of non-fighters, N_{Fe} = number of females, $c = 8.23$, θ=26.14 (after equation 2.2).

Equation 2.4 shows the number of eggs one fighter can achieve per day if in competition with other fighters and non-fighters. Using the data from table 2.9 and the numbers of fighters, non-fighters and females from table 2.1, one can calculate the $N_{F,eggs}$ initially and after the time period of one week, when fighters cannot kill other males of its own cohort. The initial estimate of fighter paternity arises from the initial densities (and so is an estimate of paternity if no non-fighters are killed by the fighters). The final estimate of fighter paternity represents the paternity that occurs after fighting-related mortality (which occurs in the first days prior to hardening of the cuticle). Comparison of the two measures estimates the fecundity benefits due to fighting.

The result of the above calculations can be found in figure 2.3 and 2.4 and table 2.8.

t	*Median*	n
0	2.6	18
1 week	6.0755	18

Tab. 2.8: *Median fighter fecundity (eggs female^{-1} day^{-1} over densities 5, 10, 20, 50 at start of experiment; therefore without the influence of any fights) and after one week after cuticle hardening when fighting ceases to be efficient to kill other males through cuticle hardening and therefore the effect of fighting ceases to exist.*

The difference in eggs per fighter per female after all fights have completed is significantly greater than the number of eggs that could have been achieved, had no fights taken place (Wilcoxon rank sum test, n=18, p<0.0001). Fighters that kill other non-fighters have, therefore, an advantage by killing off

other males and "snatching" their matings.

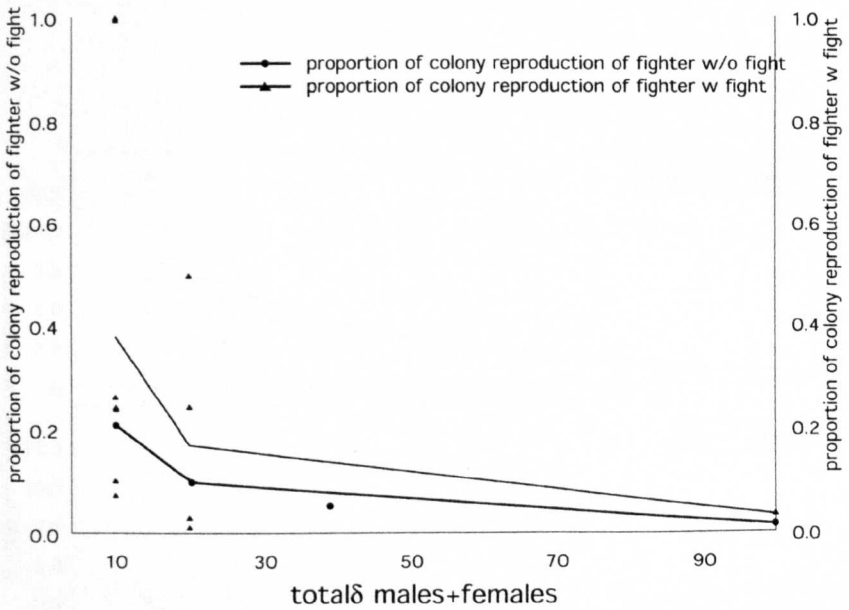

Fig. 2.3: *Proportion of colony reproduction of fighters per day vs total animal density δ initial at experiment start (initial = expected reproductive success, by frequency w/o fights, lower line) and after one week post cuticle hardening when fighting is not successful anymore (= gained reproductive effort with fights, upper line). w = with, w/o = without. Fighters gain an increase in overall colony reproductive success through fighting.*

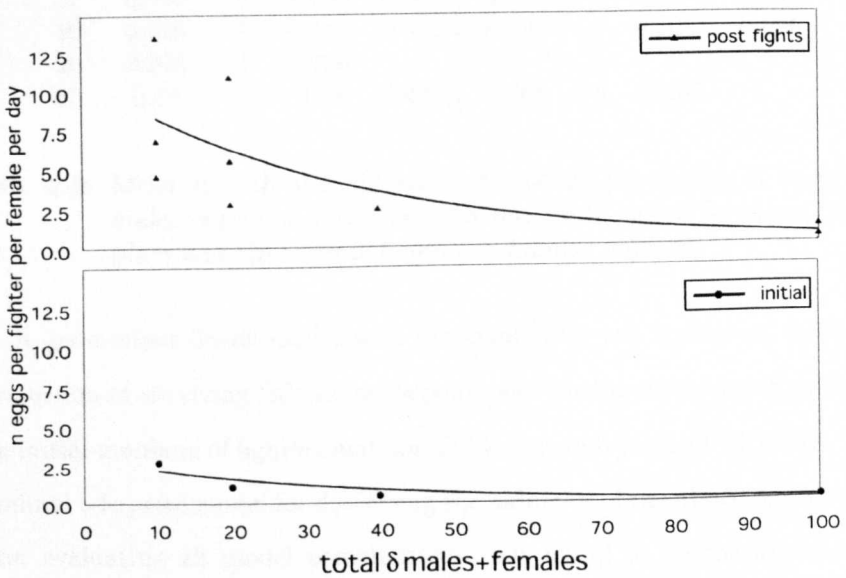

Fig. 2.4: *Fighter fecundity (eggs female^{-1} day^{-1} vs total animal density δ initial at experiment start (initial = expected reproductive success, by frequency w/o fights) and after one week post cuticle hardening when fighting is not successful anymore (post fights=all fighting took place). Each fighter has a higher chance of achieving more reproductive success through fighting.*

Survival probabilities of fighters and non-fighters

This experiment investigated survival of fighters and non-fighters over a period of a week within colonies of different densities (see table 2.9).

	fighters				*non − fighters*			
δ	Median	n	Mean	SE	Median	n	Mean	SE
5	0.550	10	0.605	0.082	0.000	8	0.1875	0.131
10	0.200	5	0.488	0.021	0.1667	4	0.1667	0.095
20	0.200	1	0.200	-	-	-	-	-
50	0.58	2	0.58	0.420	0.408	1	0.408	-

Tab. 2.9: *Mean and SE and Median and n of the percentage of surviving males vs pair density δ over five days until cuticle hardening takes place and fights do not result in killings anymore.*

A generalised linear model with binomial error was conducted with the proportion of surviving fighters as response and the log transformed values of the initial numbers of fighters and non-fighters as independent variables. The minimal adequate model for describing the influence of the above parameters, after evaluating all model combinations, was found to be the model with the log transformed number of fighters as a single term (binomial GLM, *term*=log(n of fighters), df=11, $p(\chi^2)$=0.03).

A non-fighter's chances of survival seems to rise when the proportion of fighters to non-fighters becomes smaller and density increases. In lower densities fighters managed to kill off all non-fighters (table 2.9), while in a density of 50 with one fighter present, only half of the non-fighters were killed within a week. Fighters together have a higher percentage of survival over all pair densities (1-50) and fighter densities than non-fighters (Wilcoxon rank-sum

test, n=13, m=18, $p < 0.0016$). Fighters have a survival probability of 55% while non-fighters have a survival probability of only 20% over all densities. Generally one would expect fighters having a higher risk of getting killed as they are actively seeking confrontation, but their armour and aggression seems to be sufficient to compensate for this.

2.4 Discussion

In a changing environment, phenotypic plasticity leads to changing phenotypes (Meyers and Bull, 2002). This phenotypic variation can be found within single individuals (Levins, 1968), among individuals in the population at one time (Ballare et al., 1990) or over generations (Gibson et al., 1992). With its density-dependent morph determination *S. berlesei* seems to react to changes in population density caused by intrinsic (e.g. population oscillations through migration) and extrinsic factors (e.g. temporal and spatial variation - food). In a finely grained heterogenous environment (spatially or temporally) it seems to be best for the male to delay the decision to become a fighter as long as possible. Presumably the shorter the time period is between the decision time to become a fighter or not and the time when the actual adult male emerges, the better a male is prepared for the situation he encounters. The fighter morph determination decision is possible up to the deuteronymph stage and is, as has been shown above, highly influenced by environmental conditions. Long distance dispersal, when it occurs, is via the deuteronymph (hypopus) and phoresy. Given this, the tritonymph (the stage

after the proto- or deuteronymph) may well develop on a different patch, at different population densities, than its natal patch. Conditions then may well predict the conditions it will experience as an adult. It therefore makes sense to delay the decision regarding which morph to become, as long as possible. Presumably development pathways preclude a decision at the time of the last molt, so that the decision cannot be made later in the development stages.

There is an exponential-like decline in the probability of becoming a fighter with density, rather than a step like threshold. The reasons lie most likely in a reduced fecundity for fighters in higher densities and a system inherent condition-dependence. This condition-dependence seems to be tightly coupled with the density-dependence, or simply the higher density leads to a lower level of food for all animals. Therefore the lower fighter numbers at lower densities with low food are responding to previous low food conditions, such as might occur at the time they hatched. Therefore the condition-dependence is a potential mechanism for delayed density-dependence to occur. However, even a well-fed isolated male will not develop into a fighter if olfactory clues from dense cultures are introduced to them (Timms et al., 1980). Therefore there is some true density-dependence and the condition-dependence is not the only mechanism of density-dependence.

Condition-dependence on *S. berlesei* was found by Radwan (1995). Feeding the mites on filter paper led to the same number of fighters when reared alone as well fed fighters at $density = 24$. Fighters on filter paper simply have very little to invest in extra legs. It is more astounding that actually two fighters developed out of 41 with a very low amount of food. This also

explains why here even under low food conditions (a minute amount of yeast, which still is more nourishing than filter paper) at very low densities, fighters developed with about 40% probability.

Condition-dependence seems to be of major importance in male polymorphism and has recently also been found in *Jassa marmorata* (Kurdziel and Knowles, 2002). This seems to be highly likely, as in *S. berlesei* as in *J. marmorata* and *J. falcata* the fighting or aggressive morph needs to build up extra body mass. Fighters in *S. berlesei* have to develop extra leg mass and *J. marmorata* and *J. falcata* have larger extra morphs, which need more biomass to build up. Interestingly there is also a condition-dependence in *Ontophagus spec.* where the dominant phenotype has to develop extra horns (Emlen, 1999). It seems to be therefore a major prerequisite that, for an andropolymorph to develop, the conditions have to be at least reasonable for the build up of extra body mass. It seems to be also necessary that the trade-off between the extra-body mass and reproductive benefits have to be overcome in the first instance, before one looks at general reproductive trade-offs, or the condition-dependent trade-off has to be considered much more strongly.

The fighter "gambles" on being the winner of all fights. This results in the gains being very high compared to a non-fighter as he not only gains a higher percentage of all matings and a higher egg number than a non-fighter in the same condition, but more eggs in an initial low density population. This can be more important than in an already established population, as these eggs are the genetic founders of all future generations. A fighter could potentially

gain a higher percentage of the overall female fecundity than a non-fighter, dependent on both ratio and density. At density five he could gain 100% more fecundity than a non-fighter dropping to about 50% at higher densities. A fighter could therefore achieve (also in higher densities) more fecundity, as a result of killing other rivals. The variance at lower densities (due to the random outcome of fights) seems to be substantial, as the mite might be killed at emergence or he might win all fights. Only in very high densities, the probability of gaining a high percentage of the overall colony reproductive success sinks, as there are simply too many competitors being it fighters or non-fighters. This seems to be therefore an adaptation to the extremes and a fitness to reduced variance seems unlikely, as fighters do develop at low densities. There is also an inherent relationship between population-size and variance. Given that the numbers relate to independent Bernoulli trials, the variance one Bernoulli trial (become a fighter with probability 0.9) is higher than in 100 Bernoulli trials according to the law of large numbers (in repeated, independent trials with the same probability p of success in each trial, the percentage of successes is increasingly likely to be close to the chance of success as the number of trials increases). Therefore the risks, but also the potential gain is much higher for a fighter in lower densities, while in higher densities, there is as much benefits and potential costs are reduced and the fighter's fitness will converge on the average for a male.

By actively changing the relation between himself and other individuals the fighter increases the variance and the chance to gain up to 100% of all reproductive success, if he kills off all competition. This seems to be the

reason, why fighters are also relatively often present at densities > 2. Sperm competition favouring the last to mate therefore promotes a polymorphism that kills all other males and is highly aggressive. This can be seen as a form of advanced more effective mate guarding, as it can be found for example in wasps (Field and Keller, 1993) and in birds (Westneat, 1993).

The overall results show that the effects initiating the density-dependence as mentioned in Radwan (1991) are not necessarily the only influencing factors. Radwan (1991) discusses that fighters would gain fewer matings through increased time spent fighting and that this would be the trade-off in the fighter morph determination. While this might be the case, a very high influence on the trade-off of being a fighter in high densities seems to be, that the reproductive success gained will be very low through simple frequency-dependence, the chance of being killed and a reduced female fecundity at higher densities.

3. THE LIFE-HISTORY OF THE MODEL SYSTEM

Abstract

1.) Fitness is a measure of performance across the whole life-history, so experiments were conducted to assess both the mean life-history
2.) and how it varies with differences in environmental conditions.
3.) There is considerable plasticity in the life-history, which results in changes in the vital rates with changes in food availability and density.
4.) Evolutionary models therefore have to take into account the variation in traits, and not treat them as fixed, density-independent values.

3.1 Introduction

Fitness is a property of the whole life-cycle and not any single component of it (such as reproductive success or survival) (Benton and Grant, 2000). Therefore the evolutionary costs and benefits to any life-history decision must be assessed within the context of the life-history as a whole. This is especially true where, for example, there may be plasticity in life-history traits other than the one under investigation. Fighter development is condition-dependent, and an animal's condition is likely to be influenced by both the number of competitors, their ages (adults vs juvenile) and their conditions.

The current state of knowledge regarding *S. berlesei*'s life-history has been described in the introduction of this thesis. However the focus has seldom been on detailed estimates of its life-history parameters. How long does it need for an egg to hatch? Does this alter with a change in feeding regime? How many juveniles actually survive into adulthood? This chapter, the accompanying paper (Beckerman et al., 2003) and the technical report (Beckerman et al., 2002) will provide an insight into this.

3.2 Methods

3.2.1 General methods

Populations of *S. berlesei* were collected from an agricultural muck heap in autumn 1996. The animals had been kept since in stock cultures in 24 °C incubators. Food was provided in the form of granulated yeast. These were sieved to reduce variation. One granule averages 1.5 mg ± SD 0.35. Vials for culture and experiment were glass tubes with a diameter of 20 mm and a height of 50mm. These were half filled with Plaster of Paris $CaSO_4 \cdot {}^1/_2 H_2O$. This kept humidity when the Plaster of Paris was kept damp.

The mites were monitored using a Leica MZ 8 binocular microscope and a hand held counter.

Eggs were taken from the aforementioned stock cultures. The juveniles hatched from these eggs grew up under two conditions. One with ad libitum food (balls of yeast) in low density (20 mites) and one with scarce food (granulated yeast) and high density (approx. 100 mites). This constituted

a difference in rearing and eliminated effects due to effects of uncontrolled population dynamics. Once the mites had matured, they were separated into three treatments with different densities (1, 20 and 50 pairs), each replicated eight times. Each set of eight tubes were then assigned to a two by two factorial design. Treatments were the amount of food given on a daily basis (high [five balls of yeast]/low [20 grains of powder]) and the time when feeding started (feeding on the first day or after a delay of five days). Each treatment combination was replicated twice. The pairs were used to determine the fecundity of females under different treatment regimes and the survival of males and females. Every day after pairing until the last female died, the eggs, males and females were counted. After counting the adults were transferred to fresh clean tubes, fed accordingly to their assigned feeding regime and the eggs were disposed of or kept for the experiments described below. To maintain a stable density, males from the initial treatment cultures replaced males and females that died.

Eggs laid by these animals were collected on the sixth and eleventh day to obtain eggs around the peak of female reproduction and in the declining phase of the females reproduction, to look at the percentage of juveniles hatching at different treatment conditions. Each day hatching juveniles were removed from the experimental vessels.

To look at the time to maturity under different treatment combinations, eggs were collected from the paired mite tubes on days four, five, nine and ten, to obtain eggs from the peak and decline of female reproduction. This also allowed insight into possible differences in female investment over time.

On the four days 192 tubes were put aside (24 treatment combinations * two replicates * four days).

Each of these was assigned to one of the four feeding regimes described above.

3.2.2 Data analysis methods

Parametric survival analysis in S-Plus 2000 was used to estimate half-lives. Survival analysis uses censor codes for animals which leave the experimental setup due to various reasons. Different censor codes were applied where animals did not complete the test for the parameter estimated. As no animals started the test later than other animals, or started later and left earlier, only right censoring was applied for events that were not the target event. For example, in female survival right censoring was applied to events that caused death through catastrophes, like desiccation. Normal death events were given the event code. As the ultimate goal of this chapter is to gather data for several models and there were high event sizes (> 92000 events for Juveniles, > 1100 for adult survival and > 30000 for eggs), the full interaction model (all significant terms even explaining very little deviance [a measure of the degree of fit of a statistical model compared to that of a more complete model]) was used to find the appropriate half-lives (as advised by Ken Newman, Reader in Statistics, St. Andrews University).

It is assumed therefore that all available information would give the most accurate representation of the data. Although allowing for a slight increase

of deviance (less than 3%) would not represent any biological realism (Beckerman et al., 2002, 2003), it would explain more variation in the data and would therefore fit the data better.

AIC (Akaike's information criterion is used to test the relative value of different competing models) was not used for model fitting when all interactions where significant, as it penalises for the number of parameters in the model, which could also lead to a model which would explain less variation in the data. This does not apply for cases where sample sizes are relatively low and penalisation of the number model parameters is more realistic as more sample (or measurement) error would be introduced with rising number of factors (parameters) (personal communication Ken Newman, Reader, St. Andrews University).

Survival analysis data was treated as independent samples after discussion with Dr. Terry Therneau (Mayo Clinic), Dr. Tim Benton and Dr. Andrew Beckerman.

A full biological analysis can be found in (Beckerman et al., 2002, 2003) in Appendix B. Here the models were further reduced even if significance levels were < 0.05 if deviances (explaining more variation) were very low (3%), which was deemed biologically non-significant [(Crawley, 1993), personal communication Ken Wilson]. An example of a full statistical interaction model, using all significant terms can be found in table 3.4, examples for models selecting for biological significance (not statistical) can be found in Appendix A and in Beckerman et al. (2002, 2003). So if all interaction terms were significant the models were not reduced. But if parameters and

their interaction were found to be insignificant, model reduction techniques were employed.

Although this chapter focusses more on a descriptive analysis of the population dynamics, the collecting of half-lives for model parameterisation than on a detailed biological analysis, the overall analysis still entails the biological processes, just with more statistical detail.

Additionally Kaplan-Meier estimates were calculated to represent the data graphically, although this analysis was not used in any further models. Kaplan-Meier survival estimates the probability of surviving to any point from cumulative probability of surviving each of the preceding time intervals. Kaplan-Meier plots represent graphically the median probability of an individual surviving to a certain time point. Kaplan-Meier estimates are non-parametric form of survival analysis and have as such less statistical explanatory power, than parametric estimates. As mentioned above these estimates where generally used to provide a general overview over the data. The matrix model in a later chapter relies entirely on parametric estimates. Kaplan-Meier plots and estimates were calculated in S-PLUS. Kaplan-Meier estimation is based around the movement of individuals from one class to another (this is usually death, but can be hatching or recruitment to adults). The survival plots for juvenile hatching therefore represent the time to hatching, the survival times for juveniles maturation events and for adults actual death events.

3.3 Results

3.3.1 Population dynamics of a starting population

Overall 469 population days were counted. A graphical representation can be found in figure 3.1. The maximum number of eggs was reached on day 16 and was 6025. Six-hundred and ninety larvae were counted on day 16 and 563 protonymphs on day seven. The maximum number of tritonymphs of 470 was reached on day nine. The maximum number of females was 202 the maximum number of non-fighters was 175, both reached at day 16. Fighters were not born in any of the vials.

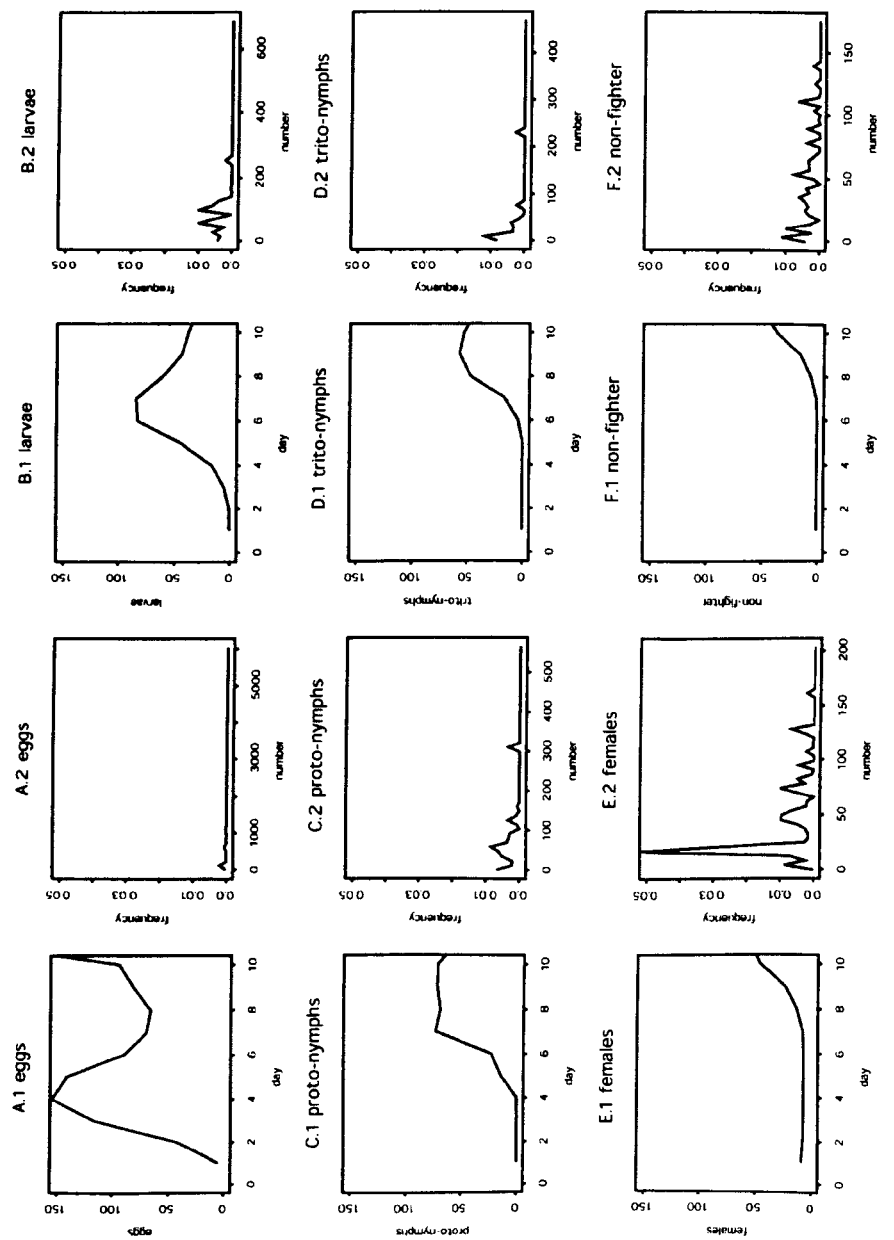

Fig. 3.1: *Friedman super kernel smooth of animal numbers and density plots of frequency of numbers of the first 10 days of 13 S. berlesei populations. A=eggs, B=larvae, C=protonymphs, D=tritonymphs, E=females and F=non-fighters. This pooled data (from high and low food cultures) originates from a separate experiment where all animals remained in a culture.*

3.3.2 Sex ratio

In the experiment 13472 females hatched and 13771 males. This is not a significant difference ($\chi^2 = 1.652$, $df = 1$, $p = 0.199$). The null hypothesis cannot be rejected looking for a deviation from the expected outcome of 50% males and 50% females. The sex ratio seems to be 1:1.

3.3.3 Female fecundity

Results over the factors can be found in table 3.1. Egg laying occurred over a minimum of six and a maximum of 33 days, depending on conditions. The daily fecundity ranged from 65.909 ± SE 6.92 eggs per day in high feeding conditions with well fed reared animals and no food delay at density one to 5.620 ± SE 0.728 in low fed, badly reared animals with five days food delay at density 50 (table 3.1), with average 14.43 ± SE 2.56 eggs per day with an inter-quartile range of 4.41-14.90 eggs.

The fecundity of females usually peaks at days four to seven (figure 3.2 lower panel). This can be influenced by delay of food, although the maximum value as with constant food provisioning (46.45 ± SE 11.18 eggs per female per day on day five) will not be reached any more (26.19 ± SE 5.80 eggs per female per day on day 12) (see Figure 3.2). This means a delay in giving food post maturation causes the peak egg production to be delayed (by the length of the delay), but overall egg production is also decreased. Catastrophic events like food depletion in the early days of maturity have a severe effect on maximum fecundity rates. With instant food absorption into resources

	$\delta = 1$			$\delta = 20$			$\delta = 50$		
z:s	$\bar{F}_{fec}$	SE	n	$\bar{F}_{fec}$	SE	n	$\bar{F}_{fec}$	SE	n
				$t = 0$					
high:high	65.909	6.972	2	14.637	1.564	2	6.850	0.724	2
high:low	26.578	5.690	2	12.921	1.701	2	8.543	1.098	2
low:high	46.263	8.895	2	12.337	1.191	2	9.570	0.988	2
low:low	30.393	4.956	2	6.840	1.114	2	7.930	0.868	2
				$t = 5$					
high:high	21.461	4.349	2	7.963	1.142	2	6.868	0.9212	2
high:low	14.000	2.417	2	7.673	1.252	2	6.895	0.780	2
low:high	21.404	3.769	2	10.825	1.466	2	5.231	0.532	2
low:low	12.111	1.820	2	5.382	1.126	2	5.620	0.728	2

Tab. 3.1: *Mean, standard error SE and number of eggs laid per female per day (F_{fec}) for densities $\delta = 1, 20, 50$, food delay $t = 0, 5$, food level z=high (ball of yeast), low (grains of yeast) and food level while growing up s=high (ball of yeast), low (grains of yeast). n=number of repetitions.*

for eggs, the maximum should be reached by day ten (day five plus five days delay), but is in fact reached only at day 12. Therefore the female should build up first her own energy requirements before investing it in the eggs. The processes for building up eggs from no resources takes approximately one to two days. The total egg output of a female in her lifetime sinks from 423.39 to 288.62 eggs.

This difference is significant (GLMM with Poisson error and log link function on the daily egg numbers, n=1125, df=1, $p(\chi^2)<0.0001$, Wald test).

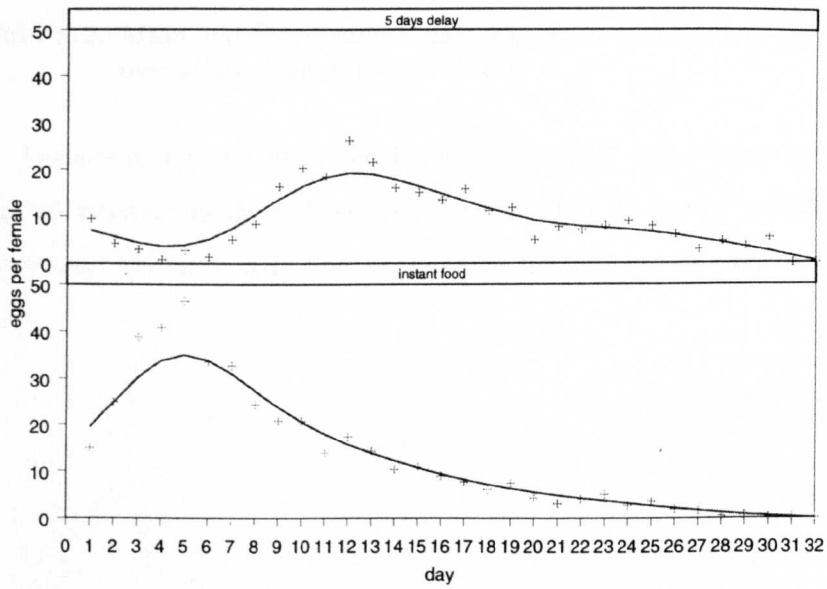

Fig. 3.2: *Number of eggs per female per day vs no food delay and five days food delay with Friedman Super kernel smooth.*

Mean and SE of the density-dependent female fecundity can be found in table 3.2 and figure 2.2. A non-linear regression on the data has been analysed in chapter 2.

δ	$\bar{x}_{dep}$	SE	n
1	26.62	1.914	576
20	10.213	0.518	528
50	7.091	0.300	521

Tab. 3.2: *Mean and SE of daily female egg production in numbers pooled over all food conditions vs density δ.*

Females in higher densities lay fewer eggs (GLMM with Poisson error and log link function on the daily egg numbers, n=1125, df=2, $p(\chi^2)<0.0000001$, Wald test) (see figure 3.3). The egg numbers fall with growing age (see figure 3.3).

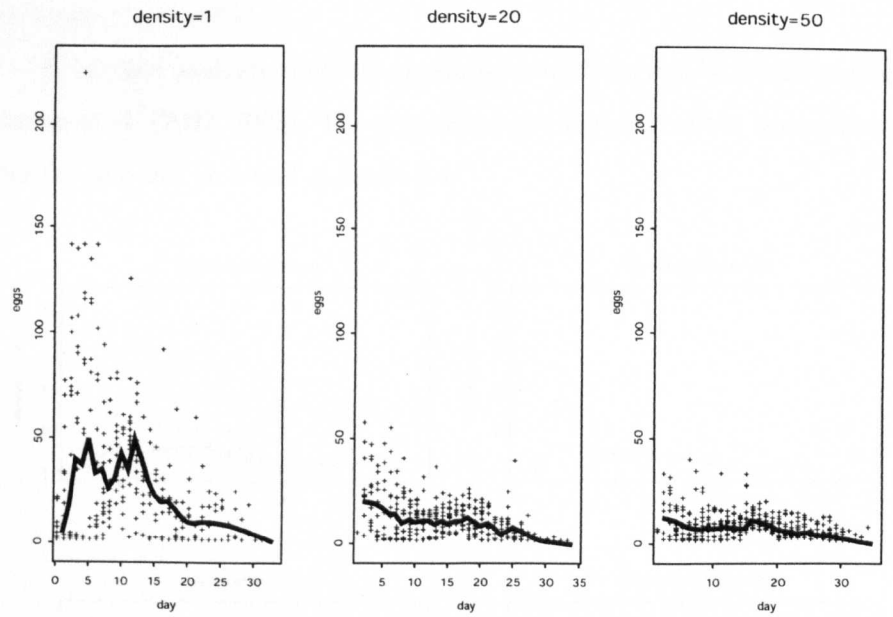

Fig. 3.3: *Number of eggs per female per day vs different densities* $\delta = 1, 20, 50$ *with Friedman Super kernel smooth. This graph represents the data summed across all factors except density, hence some of the variance is experimentally induced. For example, the double peak evident in the density=1 graph represents the effects of no delay vs a 5 day delay in food supply.*

The amount of food a female had per day showed an effect on female fecundity. Females in bad conditions laid fewer eggs than females in good conditions. This effect was strengthened by the effect of density-dependence, where females in higher densities were less fecund. A time series can be found in figure 3.4 A and B.

A detailed analysis of effects of rearing conditions can be found in Beckerman et al. (2002, 2003). The graphical representation of the time series at density one can be found in figure 3.4.

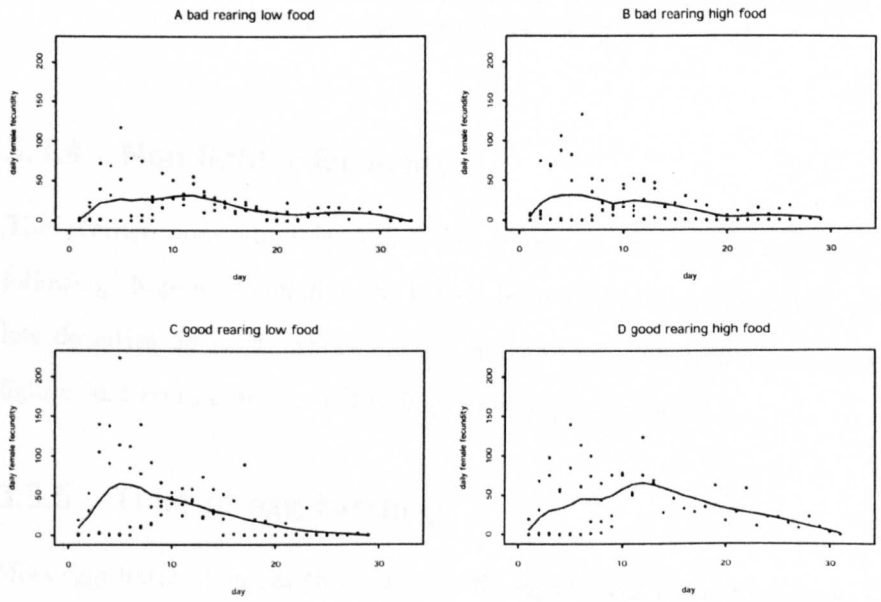

Fig. 3.4: *Number of eggs per female per day at density one with Friedman Super kernel smooths. A bad rearing conditions and low food, B bad rearing conditions and high food, C good rearing conditions and low food D good rearing conditions and high food.*

The delay of food and the interactions between rearing conditions and density and density and food delay reduced the amounts of eggs laid by females per day (see table 3.3).

	Df	F	Pr(F)
rearing conditions	1	10.9741	0.001968
adult density	1	303.3051	0
delay	1	62.325	1E-09
rearing conditions:adult density	1	21.9908	3.17E-05
adult density:delay	1	16.5656	0.000215
Residuals	40		

Tab. 3.3: *The minimum adequate model for rearing density, delay and food amount effects on per capita fecundity ($R^2 = 0.91$).*

3.3.4 Non-fighter fecundity

The average non-fighter fecundity will be set in the matrix model of the following chapter as equaling the female fecundity (see section 3.3.3), unless low densities do exist, where fighters are present. The difference between fighter and non-fighter fecundity is presented in section 2.1.1.

3.3.5 Time to egg hatching

Most eggs hatched on day three or four. No eggs hatched after nine days. Low food rearing conditions and five days food delay led to about one day delay in some vials (table 3.5). Interestingly eggs from higher densities hatched slightly earlier (figure 3.5 A). Low rearing conditions caused eggs to hatch

later on day one and two, while on day three the situation was reversed. After day four no difference could be established (figure 3.5 B).

The half-lives (here the median time until hatching) of the survival regression model with a logistic distribution are presented in table 3.5. The lowest half-life can be found with poorly fed animals at density one with five days food delay and bad rearing conditions, while one of the highest half-lives can be found at the same conditions, with the only difference that the animals were fed instantly (table 3.5). This result is surprising, therefore it has been double checked for accuracy and the underlying distribution was changed for testing purposes from logistic to Weibull which was the second best fitting distribution. Although the values changed slightly, the lowest value was again found at the same position. This seems to be therefore an accurate representation of the reality. The analysis of deviance table for the logistic survival regression model can be found in table 3.4. Relatively low half-lives for eggs can also be found at the same combination (low food, badly reared parental generation and five days food delay) at higher densities. This could be some kind of compensation for very bad conditions or having fewer resources results in less time for the actual biochemical reactions to take place, as simply less biomass has to be developed.

	Deviance	Resid. Df	$Pr(\chi^2)$
NULL		2	
DENSITY	190.726	3	0.000000000
SOURCE	1063.950	4	0.000000000
DELAY	103.974	5	0.000000000
FOOD	9.511	6	0.002042706
DENSITY:SOURCE	167.938	7	0.000000000
DENSITY:DELAY	21.512	8	0.000003515
SOURCE:DELAY	143.956	9	0.000000000
DENSITY:FOOD	114.792	10	0.000000000
DELAY:FOOD	19.467	11	0.000010237
DENSITY:SOURCE:DELAY	8.144	12	0.004321195
DENSITY:DELAY:FOOD	75.208	13	0.000000000
SOURCE:DELAY:FOOD	768.675	14	0.000000000
DENSITY:SOURCE:DELAY:FOOD	60.627	15	0.000000000

Tab. 3.4: *Analysis of deviance table of the full interaction logistic survival regression model for half-lives of egg hatching rates. DENSITY=density of animals, SOURCE=condition of rearing, DELAY=timing of first food provided, FOOD=rearing conditions. The table shows, that all parameters and their interactions explain variation in the data.*

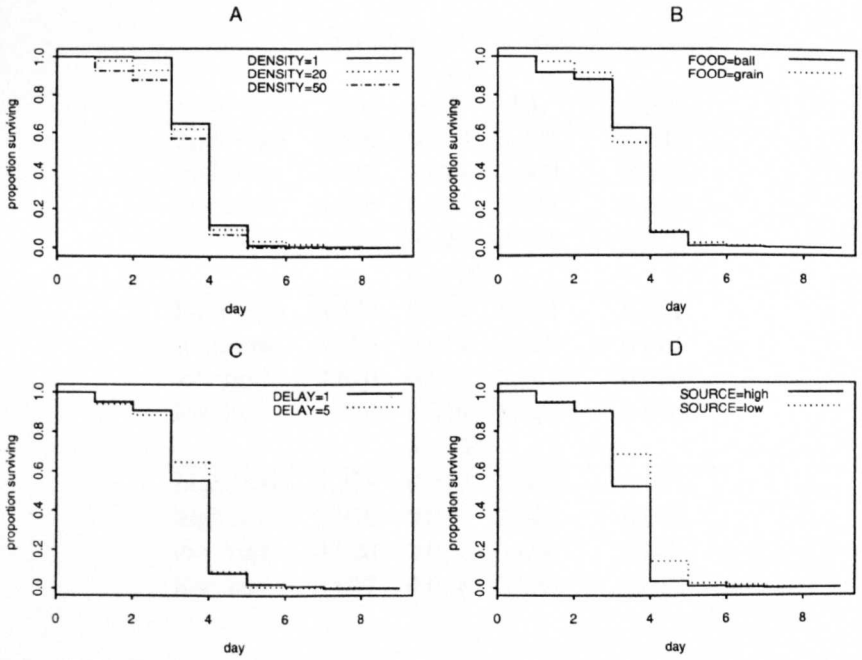

Fig. 3.5: *Kaplan-Meier survival curve estimates (predicted median survival times) of the proportion of eggs hatching vs different experimental strata. The "death" event was assumed to be hatching. A: density B: feeding regime ball = high, grain = low, C: Time of initial feeding D: Feeding regime while rearing of the mothers of the eggs.*

	fed at start		5 *days food delay*	
		$\delta = 1$		
$z : s$	HL	$\pm SE$	HL	$\pm SE$
high:high	3.529	0.023	3.607	0.039
high:low	3.718	0.024	3.909	0.036
low:high	3.956	0.023	3.943	0.035
low:low	3.852	0.027	2.785	0.062
		$\delta = 20$		
high:high	3.470	0.014	3.491	0.022
high:low	3.806	0.014	3.899	0.021
low:high	3.670	0.013	3.847	0.020
low:low	3.775	0.016	3.097	0.038
		$\delta = 50$		
high:high	3.378	0.012	3.308	0.021
high:low	3.946	0.015	3.885	0.014
low:high	3.220	0.011	3.696	0.017
low:low	3.652	0.014	3.589	0.017

Tab. 3.5: *Estimated half-lives (HL) and standard error (SE) of a logistic survival regression of eggs hatching vs density δ, at different levels of food while alive z =high (ball of yeast), low (grains of yeast) and the rearing food level of the parental generation s =high (ball of yeast), low (grains of yeast) and different start of feeding times, food given from start on and with five days food delay.*

3.3.6 Survival of eggs & juvenile hatching rate

The survival rate of eggs was over 99%. Out of 33962 eggs, an estimated ten did not hatch. Therefore eggs are not laid with insufficient resources to allow development into a juvenile.

3.3.7 Survival of juveniles

Overall, the survival analysis was made on > 9200 juvenile events. Recruitment percentages ranged from 100% at low juvenile densities to 0% under some high-density conditions (inter-quartile range=3%-84%). The extremes of the experiments ranged from individuals fed *ad libitum* of food during their development (lots of food) while experiencing low densities ("good track"), to small amounts of food ("little food") in a one off pulse while experiencing high densities ("bad track"). So some animals were fed over time ("over-time") with high and low amounts of food and some animals were fed only once at the start ("now") with either high or low food amounts. Based on the highest and lowest 10% of juveniles density, 80% of those individuals experiencing the lowest densities matured, while fewer than 1% did so under the poorest conditions.

There was no parametric distribution that fitted the juveniles death events well at early failure times. The following is calculated as the number of juveniles that actually died and did not mature.

Nevertheless as many animals only started to develop after day four and to get some estimates on juvenile half-lives, the half-lives for juvenile were

calculated using a parametric survival regression with log-logistic survival curve distribution. The log-logistic distribution performs as the log-Gaussian model at lower failure times but seems to fit better at higher failure times. The estimated half-lives can be found in table 3.6.

The Kaplan-Meier estimates split after day of egg laying, feeding pattern and food amount are presented in figure 3.6. According to the non-parametric Kaplan-Meier estimates, the time age that females laid eggs (day 4, 5, 9 or 10) affects the survival of juveniles, with days 4 and 10 having on average higher survival than juveniles from eggs laid on days 5 and 9 of adult life. Batches five and nine survival times lie lower than the extreme values. In bad conditions it matters when a female lays her eggs, eggs laid later in life have a longer survival time (see figure 3.6 A and table 3.6). A high amount of food heightened the probability of survival for juveniles slightly in mid-ranges (see figure 3.6 B). The strongest effect on juvenile survival seems to be the way in which the food is provided. Animals given their food over time matured much earlier than animals fed at the start (see figure 3.6 C).

	$batch = 4$		$batch = 5$		$batch = 9$		$batch = 10$	
$z : o$	HL	$\pm SE$	HL	$\pm SE$	HL	$\pm SE$	HL	$\pm SE$
high:overtime	13.57	0.005	10.47	0.006	9.61	0.005	9.40	0.007
high:beginning	10.70	0.007	14.21	0.004	17.08	0.004	17.88	0.005
low:overtime	8.91	0.007	8.95	0.006	9.13	0.005	9.17	0.007
low:beginning	11.95	0.005	12.51	0.004	15.01	0.004	15.71	0.005

Tab. 3.6: *Half-lives (HL) and standard error (SE) of parametric log-logistic survival regression of juvenile survival vs levels of food z =high, low and o =feeding pattern: The same amount of food was given once at the beginning or spread over the whole life time (overtime), split into different batches indicating the day of the female life time after maturation, the eggs were taken from the female.*

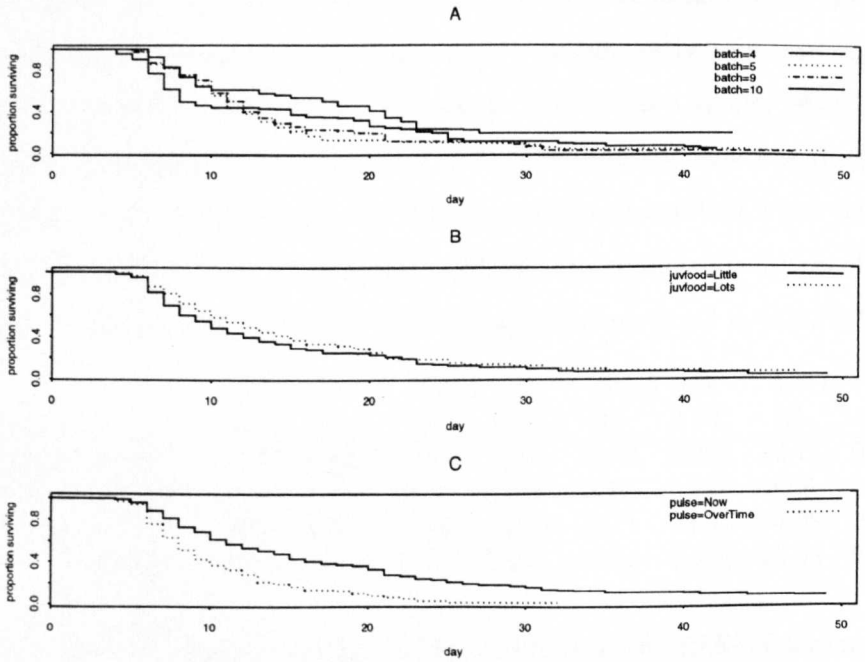

Fig. 3.6: *Kaplan-Meier survival curve estimates (predicted median survival times) for juvenile survival, split into A) batch number (indicating the day of the female life time after maturation, the eggs where taken from the female) B) food amount given, c) feeding pattern (Now=at beginning of experiment (in bulk), Overtime=over the whole experiment time).*

3.3.8 Adult maturation

The time to adult maturation best fitted a log-logistic distribution. In figure 3.7 one can see that there is nearly no difference in male and female time to maturation. As may be expected supplying resources affects the time to maturation. A pulse of food at hatching, if it is sufficiently large, can supply food to allow fast growth and maturation, and so has a similar effect to a daily large supply of food (figure 3.7 C-F). The situation regarding the time of life the adults laid the eggs for the new emerging generation is not clearly distinguishable alone from graphical analysis. The predicted half-lives of the fully parameterised parametric survival regression are found in table 3.7.

	$batch = 4$		$batch = 5$		$batch = 9$		$batch = 10$	
$z : o$	HL	$\pm SE$	HL	$\pm SE$	HL	$\pm SE$	HL	$\pm SE$
high:overtime	14.60	0.005	13.04	0.005	14.08	0.003	12.74	0.004
high:beginning	9.84	0.011	15.83	0.016	8.52	0.015	8.66	0.019
low:overtime	14.06	0.005	16.42	0.006	15.51	0.004	14.98	0.005
low:beginning	16.46	0.014	10.23	0.029	10.21	0.036	13.58	0.035

Tab. 3.7: *Estimated half-lives (HL) and standard error (SE) of parametric log-logistic survival regression of adults maturing vs levels of food z =high, low and o =feeding pattern: The same amount of food was given once at the beginning or spread over the whole life time (overtime) and the days eggs were laid in parental generation: 4, 5, 9, 10.*

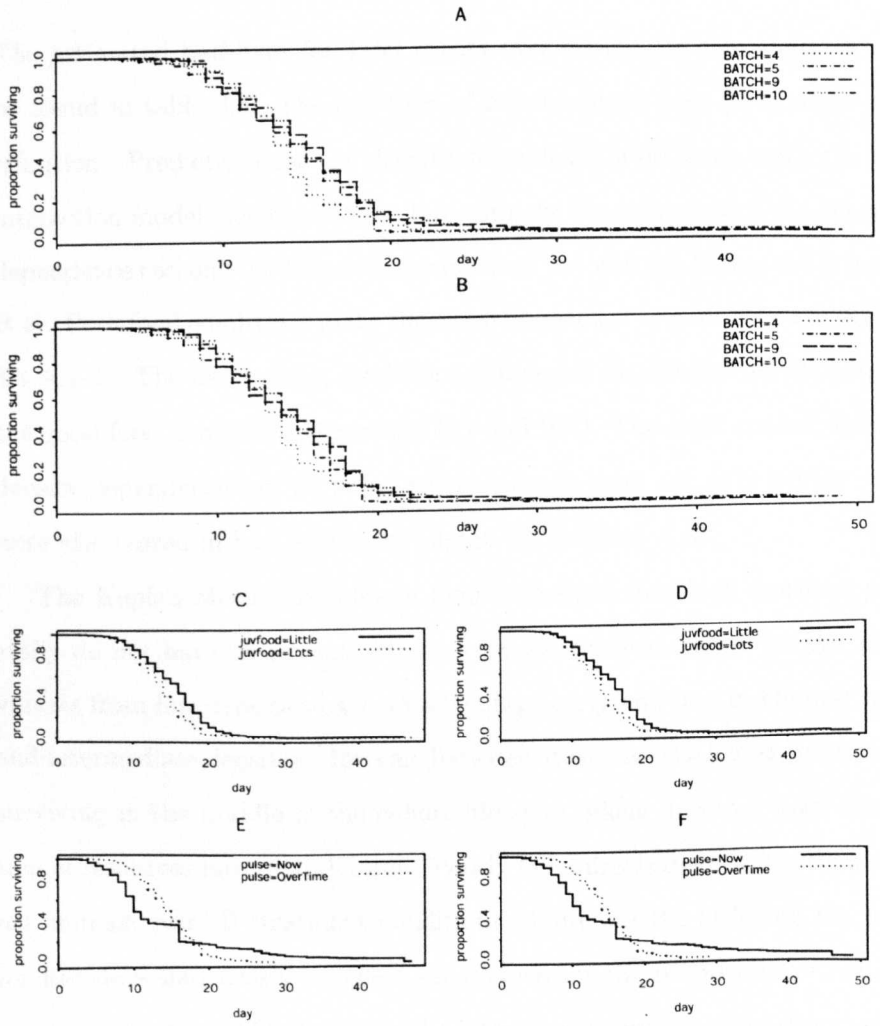

Fig. 3.7: *Kaplan-Meier survival curve estimates (predicted median survival times) for time to adult maturation by sex; A, C, E = males, B, D, F = females split into batch number food amount given, feeding pattern.*

3.3.9 Female survival

The estimated half-lives for 1159 events over treatment combinations can be found in table 3.8. The half-lives of females fitted best an extreme distribution. Predictions for the density-dependence were made with the full interaction model (see figure 3.8). Interestingly the tailing off of the density-dependence can only be found in animals that are well fed (figure 3.8 B.2 and B.4). Poor food conditions make the curve fit concave or more concave (figure 3.8 A.1-4). The bad rearing conditions dampened the density-dependence of the good food conditions (figure 3.8 B.1 and B.3). The most convex form of density-dependence can be found where animals were not only fed low, but were also reared in bad conditions (figure 3.8 A.1 and A.3).

The Kaplan-Meier estimates in figure 3.9 show that high densities generally do not have an adverse effect on female survival (figure 3.9 A). Individuals from low densities have an advantage early and late in life over high and intermediate densities. Intermediate densities have the lowest proportion surviving in the middle of the cohort life span, likely due to a high allocation of resources into reproduction. Poorly fed animals died earlier than well fed animals over all treatment conditions (figure 3.9 B). Delaying the food for five days had nearly no effect on its own on the proportion of females surviving (figure 3.9 C), but here the interactions are very important, when controlled for fecundity (Beckerman et al., 2002, 2003).

Females from poor rearing had a higher probability of survival, only between days 11 and 17 the situation was reversed (figure 3.9 D). This is likely

due to a high investment into survival, than into reproduction, when conditions are bad.

	$delay:0$		$delay:5$	
	$\delta = 1$			
$z:s$	HL	$\pm SE$	HL	$\pm SE$
high:high	15.02	0.08	18.34	0.08
high:low	15.54	0.08	12.72	0.08
low:high	15.96	0.08	15.34	0.08
low:low	8.62	0.07	15.50	0.09
	$\delta = 20$			
high:high	17.46	0.05	18.93	0.05
high:low	16.77	0.05	14.95	0.05
low:high	15.92	0.05	16.48	0.05
low:low	11.37	0.05	15.61	0.05
	$\delta = 50$			
high:high	22.15	0.03	19.90	0.04
high:low	18.91	0.03	19.29	0.03
low:high	15.84	0.03	18.47	0.03
low:low	17.57	0.03	15.79	0.04

Tab. 3.8: *Estimated half-lives (HL) and standard error (SE) of parametric survival regression on the full interaction model of females survival vs density δ, at different levels of food z =high (ball of yeast), low (grains of yeast) and rearing food level of the parental generation s =high (ball of yeast), low (grains of yeast) and different start of feeding times, food given from start on and with five days food delay.*

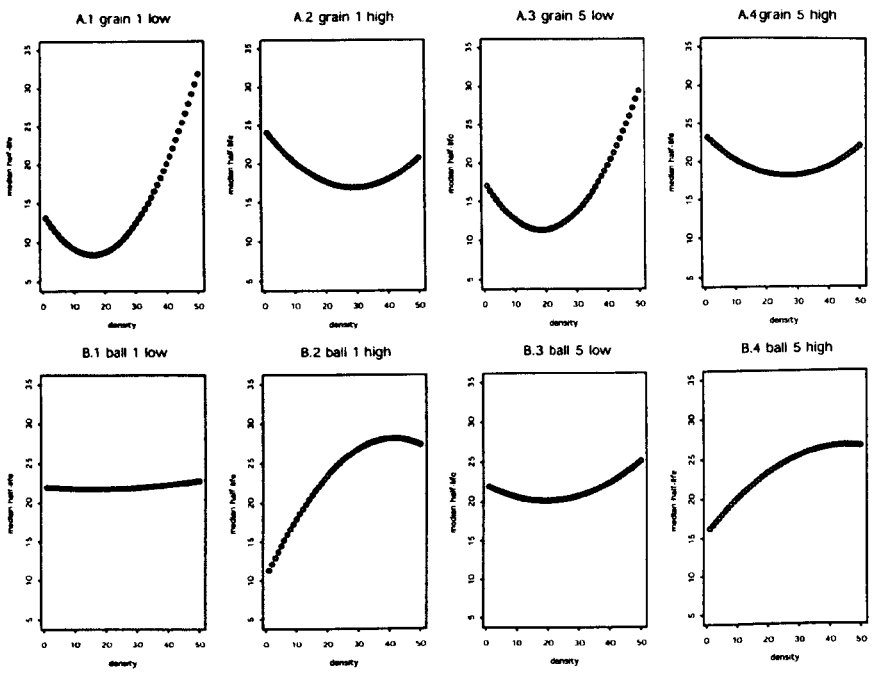

Fig. 3.8: *Female predicted half-lives by full parametric interaction model of density over different treatment combination vs total pair density, A.) first row low rearing conditions, B.) second row high rearing conditions 1.) no food delay, 2.) no food delay, 3.) five days food delay,4.) five days food delay.*

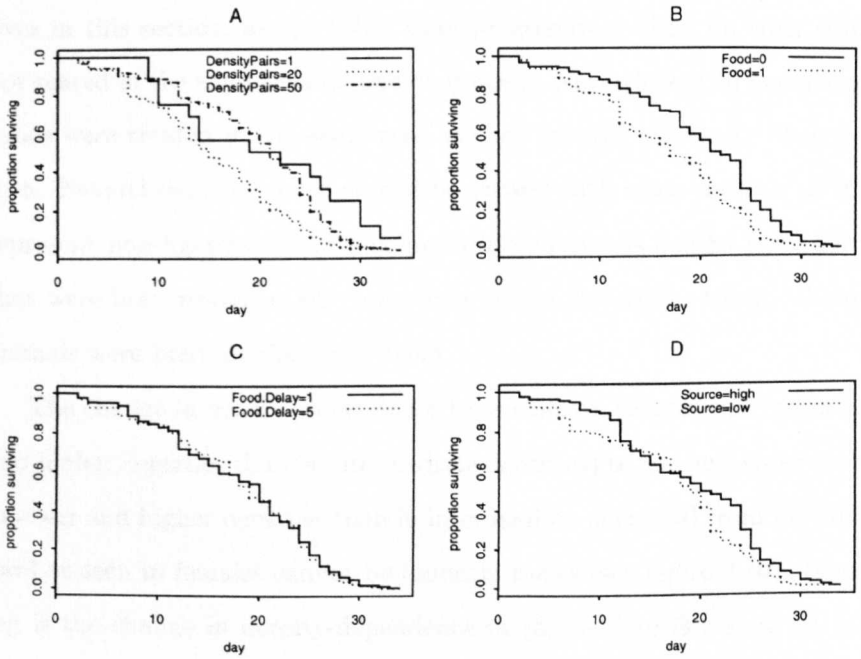

Fig. 3.9: *Kaplan-Meier survival curve (predicted median survival times) estimates of the proportion of females surviving vs different experimental strata. A: density B: feeding regime 0 = high, 1 = low, C: Time of initial feeding D: Rearing conditions.*

3.3.10 Non-fighter survival without fighter presence

In the experiments outlined above, in order to maintain the experimental densities, as females died, males were added (who had been reared under the same conditions). One can therefore not take the absolute numbers of half-lives in this section, as the tubes were progressively filled up with animals not reared in the experimental test containers. Nevertheless all experimental tubes were treated in the same way, so that relative effects are likely to be seen. Nevertheless the result should be viewed with some caution, as it will represent non-fighters out of a cohort and continuous deaths from animals that were born nearly at the same time, out of the source tubes, where the animals were bred for this experiment.

The change in density-dependence from concave (higher survival in lower and higher densities than in intermediate densities) to convex (lower survival in lower and higher densities than in intermediate densities) from low to high food as seen in females cannot be found in males (see figure 3.10). Interesting is the change in density-dependence in (figure 3.10 B.1 B.2). It is not immediately clear how bad rearing conditions would have an opposite effect on male survival that are well fed. Full investment into reproduction might be a cost with a strong trade-off as a reaction to the immediate past history, while males that are in the same situation, but are fed with a food delay would not react to an improvement in general living condition. Interestingly B1 (in figure 3.10) is the situation where the difference between low and high fed rearing becomes the strongest.

	Deviance Resid.	Df	$Pr(\chi^2)$
DensityPairs	32.8080	3	0.000000010
Food	47.4620	4	0.000000000
DensityPairs:Source	9.9993	5	0.001566014
Source:Food.Delay	116.8789	7	0.000000000
DensityPairs:Food:Food.Delay	29.9900	9	0.000000307
Food:Source:Food.Delay	9.3102	11	0.009513065

Tab. 3.9: *Analysis of deviance table for half-lives of males from the minimum adequate regression model with extreme distribution. DensityPairs=density of animals, Source=condition while being reared, Food.Delay =timing of first food provided, Food=rearing conditions.*

The minimum adequate model can be found in table 3.9. In two of the interaction terms food conditions and source conditions have an influence on the survival of males. It seems therefore to be apparent that males are under the effect of bad rearing conditions.

In particular rearing conditions and food delay seem to play a major role. Reproductive allocation, even in males, may be a mixture of capital and income expenditure so that rearing conditions, and conditions in early adulthood affect the trade off between reproductive expenditure and investment in survival.

So it seems that the sperm allocation is very important when conditions have been bad and if a food source is found and all energy is allocated towards reproduction. This behaviour is likely to be favoured in evolutionary terms, as it would result in a scenario where all is done to overcome previous bad streaks. Females in the same situation (see figure 3.8) have interestingly the weakest to non-existent density-dependence. This could mean that daily egg production is generally low at high densities (see table 3.2) and could be a reason for the u-shape of the density-dependence.

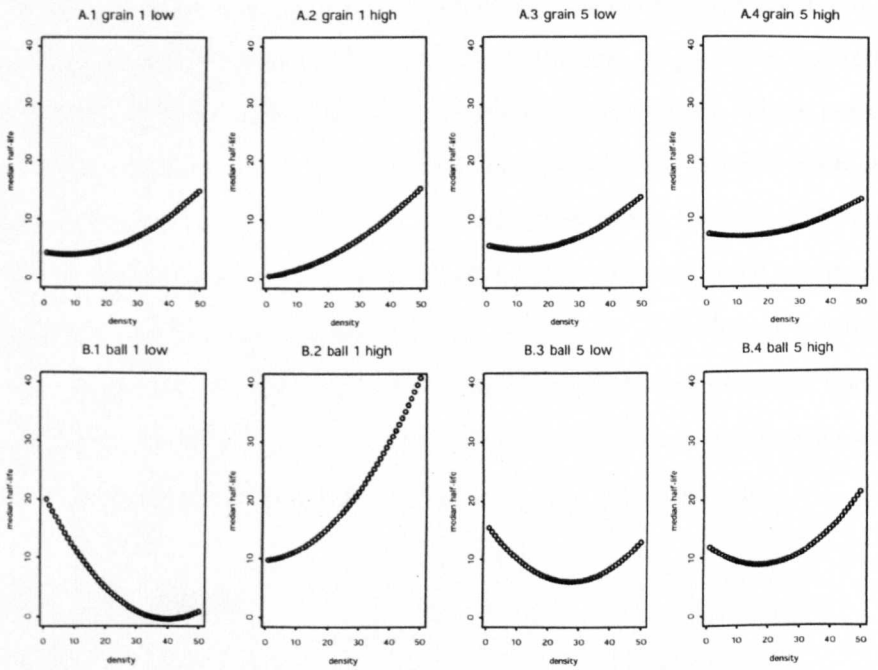

Fig. 3.10: *Male predicted half-lives by full parametric interaction model of density over different treatment combination vs total pair density, A.) first row low rearing conditions, B.) second row high rearing conditions 1.) no food delay 2.) no food delay 3.) five days food delay 4.) five days food delay.*

3.3.11 Deviation of the expected binomial distribution of male : female combination at the density of two animals

When eggs mature it might be possible, that animals adapt their sex according to the frequency of their or the other sex. Eggs were isolated in pairs, fed on balls of yeast and reared until they were adults. Three possible pair wise combinations of sex were possible: male/male, female/female, and female/male were recorded. The expected ratio would be 25% male/male, 25% female/female and 50% female/male tubes. The expected numbers of tubes, out of 72, would therefore be 18, 36 and 18. The observed numbers were 14, 44 and 14. The difference between expected and observed was not significant (χ^2=3.55, df=2, p=0.169). There is therefore no evidence that *S. berlesei* can adapt their sex according to their surroundings.

3.4 Discussion

Fitness is a property of the whole life-cycle (Benton and Grant, 2000), so understanding the life-cycle of a species is important. Conditions experienced by one life stage influenced the plastic expression of traits at subsequent time steps throughout the entire life-cycle. As a result, density-dependence was complex and highly contingent on current and past environmental conditions. Cohort effects arise throughout the life-cycle indicating that age structure in *S. berlesei* can interact with density and stochastic variation in

the environment to generate delayed life-history effects. As these delayed life-history effects are linked to density-dependent traits, it is possible that delayed density-dependence can arise in *S. berlesei* populations. For the fighter morph, developing in low densities and in good food conditions, this means that the offspring produced will benefit from the positive effects of breeding in these conditions also in the next generations, adding therefore to an advantage in his density-dependence. Density-dependent morph determination seems therefore to be coupled with the overall density-dependent life-history of the whole species.

Females typically lay more eggs under good conditions (high food, low densities) than under poor conditions. Survival also changes with condition, in a complex way, related to reproductive output. Females under the best and worst conditions survive for the shortest time (probably due to a high reproductive investment under good conditions, and starvation under poor). With medium conditions females presumably do not have enough resources to be maximally fecund, so may invest both in survival and reproduction, thereby living longer.

A scenario with high food and low densities allows females to have the greatest fecundity, but it also allows males to potentially have a high reproductive success, fertilising the eggs. This scenario reflects also the conditions under which males are most likely to develop into, and encounter, fighters. Males become fighters when there is sufficient food to (a) develop fast and (b) grow big. These conditions match those required for females highest reproductive success. The short development time, coupled with the high

fecundity, suggests that fitness will be greatest. Conversely, if there was no condition-dependence, males may mature at a time that females were not able to lay many eggs, so the fighters would be risking a lot for little potential gain.

DeWitt et al. (1998) proposed costs associated with plasticity. Animals have to maintain the energetic costs of sensory and regulatory mechanisms, and have excess costs of producing structures plastically (when compared to the same structures produced through fixed genetic responses). DeWitt et al. (1998) also proposed that there would be a developmental instability, as plasticity may imply reduced canalisation of development within each environment, or developmental "imprecision" and that genetic trade-off comes to effect as deleterious effects of plasticity genes through linkage, pleiotropy and epistasis with other genes. It seems as if *S. berlesei* fighters have certainly high costs in producing structures plastically. But even the juveniles and adults show plasticity in size, which is condition and density-dependent. The fighter has therefore not only condition-dependence working on its trait but also on his whole life-history and on it's potential reproductive success. Therefore his own discrete phenotypic plasticity is inevitably linked to the plasticity in many traits.

A discrete threshold (Roff et al., 1997) in condition-dependence as in the polyphenism (existence of several phenotypes in a population which are caused by environmental influences rather than different genetic types) of *O. acuminatus* (Emlen, 1999; Moczek et al., 2002) could not be discovered in the discrete phenotype nor any other trait. The condition-dependence on the

involved traits seems to be continuous. The lack of a discrete threshold of condition-dependence in the researched traits, could have an influence on the lack of a noticeable threshold in condition-dependent fighter morph determination. If reproductive success in females does not have a distinct threshold, it might not be evolutionary profitable for the fighter morph to establish a discrete threshold in condition-dependence for himself. From personal observation it is clear that the size differences in adults generally (Beckerman et al., 2002) are reflected in fighters. With less food provided, fighters become smaller and appear less often. In extreme low food situations, feeding on filter paper, the fighter morph nearly ceases to be expressed (Radwan, 1995).

The costly built-up of condition-dependent sexual ornaments like in *Carpodacus mexicanus* (Badyaev and Duckworth, 2003) and condition-dependent pheromone functions in *Tenebrio molitor* (Rantala et al., 2003) where condition-dependence is usually discussed in a framework of female mate choice (David et al., 2000; Badyaev and Duckworth, 2003; Rantala et al., 2003). The fighter seems to circumvent "ritual" (mate choice by body ornament display or pheromone output) female choice, by killing off competition. The high reproductive rates of females ensure for the fighter a high reproductive rate in females at low densities with high food, and the female will without "ritual" mate-choice get a partner that is in some sense strong enough, as he likely survived some fights. Handicap models of sexual selection predict that male sexual ornaments have strong condition-dependent expression and this allows females to evaluate male genetic quality (David

et al., 2000). It seems that this is possibly also true for non-sexual, but fighting body alterations like in beetles, mites and amphipods and many more sexually dimorphic species (Gross, 1996) that show condition-dependence at least on the polyphenic trait. David et al. (2000) found also other non-sexual condition-dependent traits but did not find an apparent link to reproduction or fitness in a sense of quantitative output (like egg numbers).

In conclusion, one can state that the overall life-history of *S. berlesei* is complex and shows elements of complex condition and density-dependence, cohort effects and maternal effects. The fighter morph can use these life-history parameters for himself, as he does when having the highest probability of becoming a fighter when females have their highest egg production. One can also deduct that the condition and density-dependence of other life-stages and the other sex enforces a density-dependent morph determination, as reproduction will play directly into the costs and benefits being a fighter morph.

The plasticity of the life-history, especially regarding changing fecundity, survival and growth rate with conditions (food and density) is overall complex, the mapping of reproductive success onto fitness is not that straightforward. As a result, modelling will be used to confirm the results that fighters develop in an adaptive situation.

4. MODELLING THE EFFECT OF STOCHASTICITY ON FIGHTER DEVELOPMENT

Abstract

Amalgamating the results from the previous experiments, the influence of stochastic population dynamics on male strategy is modelled. The results show:
1.) A clear influence of the probability of certain densities occurring on the life-history of the fighter morph development rule.
2.) With increasing probability of lower densities, becoming a fighter is more feasible.
3.) The ESS rule changes, while in a stable high density environment a density-dependent fighter rule is never selected for.
4.) This indicates an influence of stochastic population dynamics on life-history evolution.
5.) Modelling individual variation into the fighter rule indicates some buffering effect of this form of variation.

4.1 Introduction

In the previous chapters the life-history of *S. berlesei* was described. Fighters mostly develop in low densities (section 2.1.1), are affected by condition-dependence [section 2.1.1 and Radwan (1995)] and are likely to have a higher

fecundity in lower densities (section 2.1.1). Furthermore it was established in chapter 3 that condition, density, rearing effects and time of food provisioning influence the population dynamics of *S. berlesei* [see also Beckerman et al. (2002, 2003)]. Poor living conditions cannot only reduce female daily egg production [and with this life-time reproductive success (Beckerman et al., 2002, 2003)] but also severely prolong maturation time in juveniles (section 3.3.8). Density-dependence on female survival is more complex than normally anticipated. In section 3.3.9 it is shown that in fact intermediate densities do have the worst effect on female survival.

Therefore one has to see *S. berlesei*'s secondary polymorphism (negative density-dependent) (Woodring, 1969) not only in stable conditions, either good or bad, but also in different combinations of those. After all, only in these stochastic conditions, where low densities occur, secondary polymorphism (Woodring, 1969) as opposed to primary (frequency-dependent) polymorphism (Woodring, 1969) makes sense. Therefore, trivially, low densities have to occur to be taken advantage of in evolutionary terms. Low densities occur either by dispersal and colonising of a new patch after a previous patch is exhausted, or after catastrophic events or poor living conditions which lower the overall population density.

Dispersal has been shown to affect polymorphism (Jobst et al., 1999). But why do *S. berlesei* develop only secondary polymorphism and not maintain frequency-dependent polymorphism like other Acaridae, for example *R. robini* (Radwan, 1995, 1996; Radwan and Siva-Jothy, 1996; Radwan, 1997; Radwan et al., 1999; Radwan and Klimas, 2001)?

So a likely cause for secondary polymorphism, besides dispersal, may be stochasticity, as this might also ensure that low densities do occur and a density-dependent phenotype can use the benefits of this environmental condition. Nevertheless it might also be possible that stochasticity does not enhance the advantages of secondary polymorphism and dispersal alone is the only option.

To investigate the likely effect of different levels of variation on secondary polymorphism, invasibility analysis as outlined in section 1.4 and a matrix model as described in section 4.1 will be employed. The data and analysis from chapter 2 and chapter 3 will help to describe the life-history of *S. berlesei* in mathematical terms. This life-history data, taken from high and low densities, bad and good conditions, will be employed in a random fashion to help to provide an insight into the effect of stochasticity on the fighter development of *S. berlesei*.

Populations are structured. To represent this in models, matrices have been used to represent this structure mathematically (Leslie, 1945; Caswell, 2000). Leslie (1945) pioneered the matrix model approach. The basic idea is that a state vector of the density of the individuals in each stage is multiplied with a projection matrix, called here population projection matrix, which then gives the state vector at the next (time)-step. $\mathbf{N}(t)$ = number of animals at time t. $\mathbf{N}(t+1)$ = number of animals at time step + 1. $\mathbf{A}$ = projection matrix.

$$\mathbf{N}(t+1) = \mathbf{A}\mathbf{N}(t) \tag{4.1}$$

Entries in the population projection matrix can be constant or consist of entire functions. One denotes a place in the matrix with a subscript pair of x, y. Then an element in row two column five is $a_{2,5}$, whereby a is a placeholder for a term describing the event taking place at this position. The matrix is necessarily a square matrix with a dimension that represents the number of stage or age classes. In a simple case all elements in $a_{1,2}$ to $a_{1,j}$ are terms for the stage classes fertility multiplied with its probability of survival. Here the transition probabilities (sometimes fertilities) to the next stage are given with the probabilities that the individuals in this age stage actually survive to contribute to the next stage. The sub-diagonal from $a_{2,1}$ to $a_{j-1,j}$ represents the survival probabilities of the individual. Here it is simply calculated what percentages of animals do survive in this age class.

So the vector $N_{t,j}$ is the number of organisms in age class j at time t. How this vector changes with time describes the number of changes in the number of organisms in each age class as the population develops through time, following iteration of the matrix multiplication (equation 4.1). Alternatively, analytical methods [outlined in Caswell (2000)] can be used to explore the equilibrial properties of a matrix model. Matrix models operate in discrete time and are therefore an approximation to real continuous time events.

$$\begin{pmatrix} N_{1,0} \\ N_{2,0} \\ N_{3,0} \\ N_{4,j} \end{pmatrix}_{t+1} = \begin{pmatrix} 0 & F_1 & F_2 & F_j \\ p_1 & 0 & 0 & 0 \\ 0 & p_2 & 0 & 0 \\ 0 & 0 & p_{j-1} & 0 \end{pmatrix} \times \begin{pmatrix} N_{1,0} \\ N_{2,0} \\ N_{3,0} \\ N_{4,j} \end{pmatrix}_{t} \tag{4.2}$$

In these (Leslie) matrix models extra terms for stochasticity and density-dependence can be added to terms describing the individual stages through the (time)-steps.

Unlike non-stochastic models, stochastic models can only be analysed for a limited set of equations and circumstances (Tuljapurkar and Caswell, 1996). The complexity of the biological system under investigation (structured models with non-linear density-dependence and added noise) mitigates against finding analytical solutions. Instead, numerical solutions will be sought using Monte Carlo based methods.

This involves a model that accounts for a structured life-history, works for populations in density-dependent and stochastic environments and from which one can calculate invasion exponents. Additionally, the techniques required will specifically be used to model the evolution of behaviour in fluctuating environments, where the population sizes may become very small.

4.2 Methods

4.2.1 The matrix

A matrix was developed as in equation 4.3.

The entries in the matrix correspond to:

1=daily survival of eggs, 2=fighter fecundity, 3=non-fighter fecundity, 4=egg to juvenile recruitment, 5=daily survival of juveniles, 6=juvenile to fighter recruitment, 7=juvenile to non-fighter recruitment, 8=daily fighter survival, 9=daily non-fighter survival, 10=female

fecundity, 11=juvenile to female recruitment, 12=daily survival of females

Vectors N_{t+1} and N_t give the state of the system. The vector elements are N_e= number of eggs, N_j=number of juveniles, N_F=number of fighters, N_{NF} =number of non-fighters, N_{Fe}=number of females

$$\begin{pmatrix} N_e \\ N_j \\ N_F \\ N_{NF} \\ N_{Fe} \end{pmatrix}_{t+1} = \begin{pmatrix} 1 & 0 & 2\times 8 & 3\times 9 & 10\times 12 \\ 4 & 5 & 0 & 0 & 0 \\ 0 & 6 & 8 & 0 & 0 \\ 0 & 7 & 0 & 9 & 0 \\ 0 & 11 & 0 & 0 & 12 \end{pmatrix} \times \begin{pmatrix} N_e \\ N_j \\ N_F \\ N_{NF} \\ N_{Fe} \end{pmatrix}_t \tag{4.3}$$

Pulsed reproduction at the end of each daily time step was assumed. In every term a formula was entered resulting from experiments described earlier. The focus of the investigation is the juvenile to fighter recruitment (6). A trade-off between fighter fecundity and density (successful fighters monopolise females at low density) and between survival and density (non-fighters at low density are more likely to be killed than fighters) was assumed. Then the ESS decision rule is found relating the density to probability of developing into a fighter.

The model is then run for 3000 time steps to allow the population dynamics to settle on the attractor. Subsequently an invasion is simulated using an invader strategy, which has the same matrix as the resident, with only a slight difference in the probability of becoming a fighter at a given density. The invader experiences exactly the same "environment" as the resident, and the density-dependence is based on the numbers of residents only (thus the

system is linearised at the point where the invader population is zero). A least-squares regression of the logged population size of the invader against time estimates whether or not it is invading. The mean slope of the regression of a large number of replicates estimates the invasion exponent (Rand et al., 1994).

4.2.2 Half-lives to survival estimates

The median half-lives give the estimated time when 50% of all animals have died.

The survival rates for each treatment combination were estimated using the negative exponential formula equation 4.4 also cited in Bellows (1981). Using this continuous time model in a discrete matrix model has the property, that $N_0 \cdot e^{-rt}$ changes between two adjacent integers at the rate of e^{-r} so one can set $e^{-r} = \lambda$ and can calculate N_{t+1} as $N_t \cdot e^{-r}$.

Here r is the rate of population change, N_0 = current density, N = $0.5 \times N_0$ and t = half-life times.

$$N = N_0 \cdot e^{-rt} \tag{4.4}$$

$$r = -\frac{ln(\frac{N}{N_0})}{t} \; where \; \frac{N}{N_0} = \frac{1}{2} \tag{4.5}$$

This gives us the rates of change for different densities. An example of the calculated survival rate values can be found in table 4.1 using ($\lambda = e^r$) to allow survival rates to be read more easily.

Using equation 4.6, one has an estimate of the change in animal numbers each day.

$$N_{t+1} = N_t \cdot e^{-rt} \tag{4.6}$$

The calculations were done for two kinds of living conditions of the experimental animals, reflecting extreme differences in living conditions.

δ	*good track*	*bad track*
1	0.9549	0.9562
20	0.9611	0.9566
50	0.9692	0.9570

Tab. 4.1: *Estimated survival rate of females per day ($\lambda = e^r$), good and bad track at different pair densities δ.*

Calculated were a so-called "good track" and a "bad track". "Good track" means that the animals from which the data for the calculations stems, had "ad libitum" food in the parental (rearing) and in the F1 generation, and vice versa. At this point only the extreme conditions were considered, but the model leaves room for further expansion in the future.

4.2.3 Egg to juveniles transition

Egg to juvenile transition was calculated using the half-life of the eggs and calculating the number of eggs going from the egg stage to the juvenile stage. This is formulated as $N_{juveniles} = N_{eggs} \cdot (1 - \lambda_{egg-hatching})$, as it has been established that nearly 100% of all eggs survive as mentioned in section

3.3.6. Therefore one uses as daily survival rate the hatching rate as our half-lives (section 3.3.5). The number of juveniles hatching is the inverse of the computed exponential r of the half-lives for egg hatching rate as analysed in section 3.3.5.

δ	*good track*	*bad track*
1	0.1779	0.1747
20	0.1803	0.1795
50	0.1854	0.1886

Tab. 4.2: *Estimated λ of egg daily hatching rate, good track (parameters calculated from animals in good conditions) and bad track at different pair densities δ.*

4.2.4 Juvenile survival

The half-lives of the juveniles have been estimated in section 3.3.7. From this one calculates survival curves for good and bad conditions. The results for juvenile survival are found in table 4.3. Bad track juveniles survived longer, due to interactions described in (Beckerman et al., 2002, 2003).

good track	*bad track*
0.9320	0.9559

Tab. 4.3: *Estimated daily juvenile survival, good and bad track.*

4.2.5 Juvenile to female transition

The number of animals maturing is known from section 3.3.8 as is the half-lives of the juveniles that matured. Therefore the daily transition of females is approximated as $N_{females} = 0.5 \cdot N_{juveniles} \cdot (1 - \lambda_{juvenile\ maturation}) \cdot \lambda_{juvenile\ maturation}$ expresses the half-lives as estimated in the survival analysis. A death event is therefore maturation. $1 - \lambda_{juvenile\ maturation}$ is therefore the number of adults maturing. Only good and bad track was distinguished (see table 4.4), as eggs laid by adults were followed through in the numbers laid.

good track	*bad track*
0.055	0.049

Tab. 4.4: *Estimated daily maturation rate* $(1-\lambda_{juvenile\ maturation})$, *good and bad track.*

4.2.6 Juvenile to fighter transition

In equation 2.1 the density-dependent rule for fighter development was approximated as $P_f = \frac{0.69}{\delta}$ (see chapter 2).

This relationship can also be approximated using

$$P_f = (0.69^{1/E} - 0.1806 \cdot ln(\delta))^E \tag{4.7}$$

which will be used, as it gives more flexibility in defining the shape of the relationship (through the shape parameter E). In previous experiments to

those described in chapter 2 the fighter rule was determined to be:

$$P_f = (0.9^{1/E} - 0.1806 \cdot ln(\delta))^E \tag{4.8}$$

Therefore the model was parameterised with this rule as well (which we term the 0.9 strategy).

In equation 4.7 and 4.8 P_F is the probability of becoming a fighter, δ is the momentary density and E is a shape parameter. See figure 4.1 for a graphical representation. By changing the shape parameter in the model and using invasibility analysis the uninvadable fighter emergence rule in different stochastic scenarios will be investigated. This applies to good track conditions, as due to condition-dependence in bad environments, fewer to no fighters (Radwan, 1995) (see chapter 2) emerge, the P_F is set to 0 in bad track environments.

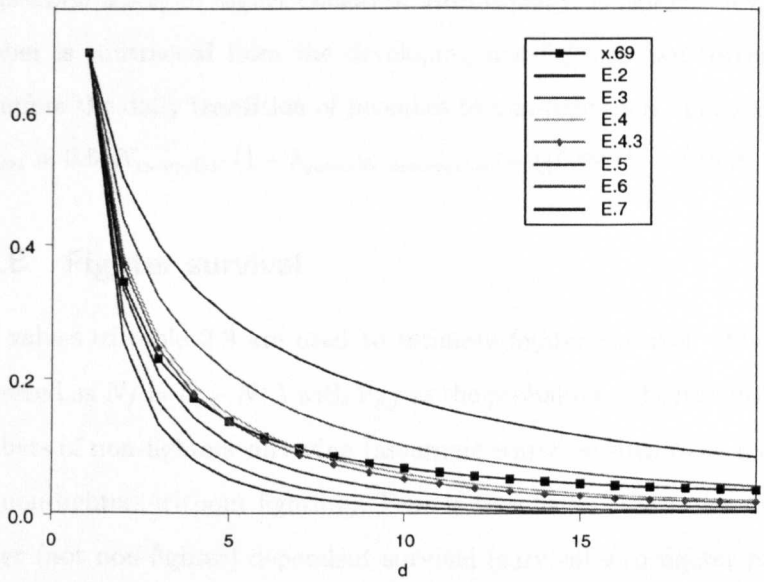

Fig. 4.1: *The relationship of the probability of becoming a fighter* $P_f = \frac{0.69}{\delta}$ *(x.69, square symbol) and a family of curves* $(P_f = (0.69^{1/E} - 0.1806 \cdot ln(\delta))^E)$ *used in the model that approximate this relationship with different shape parameter* E *values.* $E = 4.3$ *(E.4.3, diamond symbol) providing a good fit to the original relationship x.69. It is investigated here whether the shape of relationship of becoming a fighter is flexible if stochasticity is added. The shape parameter* E *is therefore the parameter under investigation, as it regulates the form of the relationship in the models presented in this chapter.*

4.2.7 Juvenile to non-fighter transition

The number of animals maturing is known from section 3.3.8 as is the half-lives of the juveniles that matured. We also know that the sex ratio is 1:1 (section 3.3.2) in higher densities. Additionally, if fighters develop, this number is subtracted from the developing non-fighters (see section 4.2.6). Therefore the daily transition of juveniles to non-fighters is approximated as $N_{males} = 0.5 \cdot N_{juveniles} \cdot (1 - \lambda_{juvenile\ maturation}) - ((0.69^{1/E} - 0.1806 \cdot ln(\delta))^E)$.

4.2.8 Fighter survival

The values of table 2.9 are used to estimate fighter survival. This can be expressed as $N_f = P_{s,f} \cdot N \cdot \lambda$ with $P_{s,f}$ as the probability of surviving and $N\lambda$ numbers of non-fighters surviving (assuming equal survival rates for fighters and non-fighters without fighting). Female survival is used to estimate non-fighter (not non-fighter) dependent survival (survival w/o fighter presence). $P_{s,f}$ is estimated as $1 - \frac{exp(\delta_f)}{10+exp(\delta_f)}$ $\forall N_f > 1$, with δ_f being fighter density and N_f= fighter number and $\forall$ = for all. Therefore a fighter's survival probability sinks with increasing fighter number, excluding density one, as one fighter does not kill itself.

4.2.9 Non-fighter survival

Non-fighter survival in the absence of fighters is modelled as the female survival in the absence of fighters. If fighters are present, the values of table 2.9 are used to estimate non-fighter survival. This can be expressed as

$N_{nf} = P_{s,nf} \cdot N \cdot \lambda$ with $P_{s,nf}$ as the probability of surviving and $N\lambda$ non-fighter survival without fighter influence. $P_{s,nf}$ will be approximated using $\frac{exp(\delta_f)}{10+exp(\delta_f)} * (1 - \frac{\delta_f}{\delta_{nf}})$ $\forall\ \delta_f >= 1 \wedge \frac{\delta_f}{\delta_{nf}} \leq 1$. $\forall\ \frac{\delta_f}{\delta_{nf}} > 1$, $P_{s,nf} = 0$; δ_{nf}= non-fighters density and δ_f= fighter density. This models when there are fewer fighters than non-fighters. In higher fighter densities (e.g. five fighters ten non-fighters) a non-fighter's survival chances will rise, as fighter's aggression towards themselves makes them more likely to attack each other and simply cannot kill as many non-fighters. Given a state where there are more fighters than non-fighters a non-fighter's survival is assumed to be zero, as fighters quite easily overwhelm a smaller number of non-fighters.

4.2.10 Egg and female survival

The density-dependent survival of eggs and females can be seen in figure 4.2. While egg maturation is negatively density-dependent (figure 4.2 A + B), females in good conditions survive longer in higher densities in good track conditions (figure 4.2 C), while there is nearly no density-dependence in bad track conditions (figure 4.2 D). The density-dependence of egg and female survival in the model was approximated using a linear regression on actual data on survival. Figure 4.2 suggests a more linear relationship of the pooled data used here, than a more complicated negative exponential, Ricker or other highly complex density-dependent model [e.g. Maynard Smith and Slatkin (1973), Hassell (1975), Ricker (1975) and Beverton and Holt (1993)], which would instill a more artificial density-dependence than the one actual

happening (see chapter 3). A simple negative exponential ($r = -0.008$) density-dependence was added for densities over 100 so that adult densities were not unbounded. Adult densities that exceed 100 by a lot are rare. The estimated values for the linear regressions can be found in table 4.5.

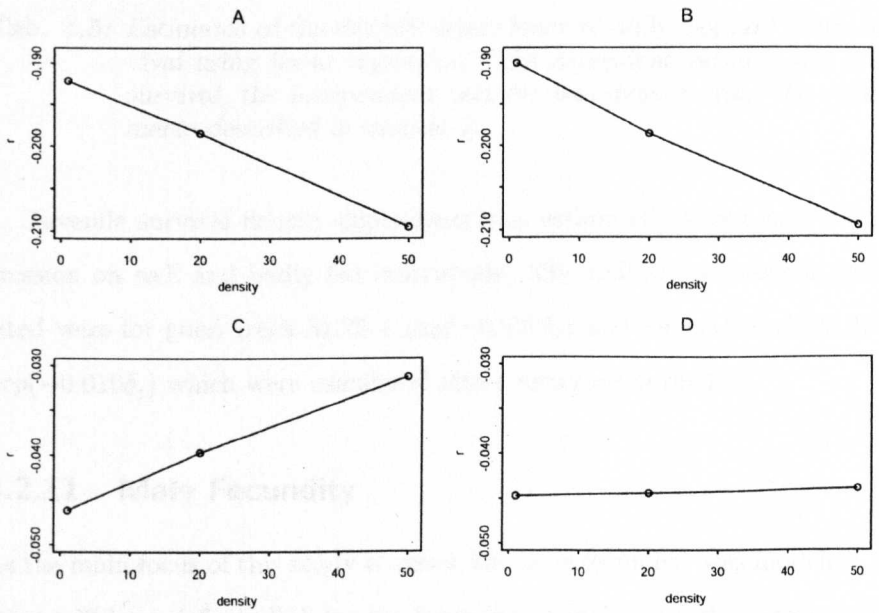

Fig. 4.2: *Density-dependent relationship of survival for adult survival and daily egg survival, A) egg good track B) egg bad track C) adult good track D) adult bad track.*

type	*good track*			*bad track*		
	Value	SE	p	Value	SE	p
egg (Intercept)	-0.1917	0.0003	0.0008	-0.1900	0.0007	0.0025
egg (λ)	-0.0004	0.00003	0.0145	-0.0004	0.00009	0.0388
adult (Intercept)	-0.0462	0.0005	0.0068	-0.0447	0.000001	0.000008
adult (λ)	-0.0003	0.00007	0.0337	-0.00002	0.00002	0.0029

Tab. 4.5: *Estimates of the density-dependence of daily egg and adult survival using linear regression. The dependent variable was daily survival, the independent variable was density from the experiments described in chapter 2.*

Juvenile survival density-dependence was estimated using a survival regression on well and badly fed individuals. The half-life regressions calculated were for good track $31.28 + exp(-0.020\delta_j)$ and for bad track $25.27 + exp(-0.010\delta_j)$ which were calculated into r using equation 4.5.

4.2.11 Male Fecundity

As the main focus of this study is males, the male fecundity was modelled, as there will be a definite link for the fecundity of males and the presence and absence of fighters. This will be modelled as the proportions of the measured female fecundities. This means that although female fecundity is modelled, the amount attributed to either fighters or non-fighters is split according to their density and their respective trade-off. As a consequence the model concentrates on the reproductive success of the males. This is based on last to mate sperm success as it exists in *S. berlesei*. Therefore the survivors will get all the benefits.

4.2.12 Female fecundity

Density-dependent daily fecundity in good conditions can be approximated by Fec_{fe}=8.23+$\frac{26.14}{\delta}$, with δ=pair density and Fec_{fe} = female fecundity. The mean fecundity per animal vs density per day (see chapter 3, table 3.2) was approximated using a non-linear regression (see table 2.7 and figure 2.2) with the response fecundity (eggs per animal per day) as represented in equation 2.2 ($y = c + \frac{\theta}{\delta}$). The proportion of female fecundity between good track and bad track is in low densities one third and in high densities one (see table 3.1). This relationship can be approximated as $1 - e^{-0.08\delta}$, with δ=pair density, which will be employed in bad conditions. Therefore in this model it is assumed that animals in bad conditions lay $\frac{1}{3}$ of the eggs of a female in good conditions.

There should be a link between the fighter phenotype and population dynamics. So if there are all fighters at high densities, there must be a trade-off between male strategy (of being a fighter) and fecundity. This means simply that fighters incur a cost of being a fighter in higher densities, as demonstrated in chapter 2. Therefore one introduces a penalty in fighter fecundity to model this trade-off. This is modelled as a multiplier and the inverse of their probability of becoming a fighter P_f, therefore $(1 - P_f) * \delta_{all\ animals} * Fec_{fe}$, which approximates the observed changed in female fecundity in different densities.

Therefore, as discussed in chapter 2, fighters can monopolise females at low densities, but as density increases, they spend more time fighting than

mating. Hence at high densities non-fighters will outperform them reproductively. This is modelled as $(P_f) * \delta_{all\ animals} * Fec_{fe}$. So fecundity is linked to the probability of becoming a fighter.

4.2.13 Stochasticity

Two extreme environmental conditions are modelled, good and bad. The environment condition is ϵ. The stochastic element κ is drawn from a uniform distribution between 0 and 1. Then a threshold value σ is introduced. σ was chosen from 0.1 to 0.99. When κ was bigger than σ ($\kappa > \sigma$), ϵ was set to good conditions. If it was smaller, ϵ was set to bad conditions (so the bad track environmental values were set). If σ is 0.5 good and bad conditions are equally likely to occur on the long run = E(ϵ).

If $\sigma = 0.1$ was set the good conditions have overall a 90% chance of occurring, this will intrinsically mean that longer periods of good conditions are likely, the reverse will happen at $\sigma = 0.9$, when periods of bad track environmental conditions are more likely. Therefore a threshold value σ was selected non-randomly and then at each time step a value from a uniform distribution was drawn (κ). The external stochastic switching of two deterministic states of a matrix has the same effect as introducing the stochasticity intrinsically in the formulas within the matrix (personal communication Dr. Simon Wood, Reader in Statistics, St. Andrews University), but see Kaye et al. (2001).

4.2.14 General model dynamics

The model was constructed using high and low food conditions as two extreme matrices. A 300 day model run for both extreme matrices can be found in figure 4.3 A & B. A model run with 50% chance of a good or bad day occurring can be found in figure 4.3 C and a model run with a 10% chance of a good day occurring can be found in figure 4.3 D.

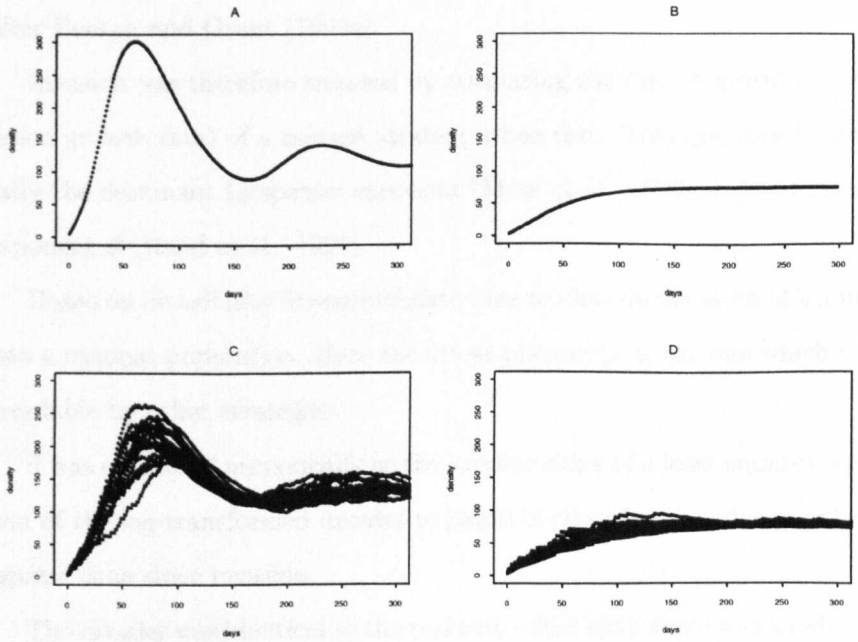

Fig. 4.3: *A) Good track matrix model run of, B) Bad track matrix model run C) stochastic run with 0.5 probability of good or bad day, D) 0.9 probability of a bad day. density vs days.* $N_{Adults} + N_{juveniles}$ *vs time.*

4.2.15 Invasion analysis and elasticity analysis

The invasions for the matrix models in density-dependent and stochastic environments were calculated as described in Grant (1997) and Benton and Grant (1999b). Invasions were estimated numerically after 3000 days (after population stability) and an invasion length of ten days using 10000 simulations with 97.5% of the original values for the stochastic runs, using values as described in Benton and Grant (2000). Elasticity analysis was performed after Benton and Grant (1999a).

Invasion was therefore assessed by estimating the rate of spread (= population growth rate) of a mutant strategy when rare. This quantity is technically the dominant Lyapunov exponent (Metz et al., 1992), termed invasion exponent ϑ (Rand et al., 1994).

Based on invasibility fitness estimates one models the invasion of a mutant into a resident population. Here the fittest phenotype is the one which is not invadable by other strategies.

ϑ was estimated numerically as the average slope of a least-squares regression of the log-transformed invader population size of an invader population against time since invasion.

The invader was identical to the resident, other than there was a reduction in the magnitude of E (which measures the steepness of the decline in $p(F)$ with density): $E' = 0.975E$. Where a slight change in E termed E' did not let an invader population grow or decline into the resident population, one would have a convergent stable strategy. The outcome of evolution will be

the strategy that can invade all others but is not itself invadable.

4.3 Results

The model results show clearly (see figure 4.4 and 4.5) that stochastic population dynamics make a density-dependent fighter strategy "worthwhile". All attractors were globally uninvadable and were therefore not local attractors. When there is a 0% to 30% chance (fighter rule $P_f = (0.9^{1/E} - 0.1806 \cdot ln(\delta))^E$ further called 0.9 Strategy) of bad times a fighter strategy never invades (see figure 4.4).

value	element	*value*	element
0.11925	egg survival	0.00234	fighter fecundity
0.1281	non-fighter fecundity	0.00253	female fecundity
0.1235	hatching	0.2711	juvenile survival
0.001	juvenile to fighter	0.000123	fighter survival
0.0074	juvenile to non-fighter	0.2087	non-fighter survival
0.00087	juvenile to female	0.1352	female survival

Tab. 4.6: *Elasticity analysis of matrix model using fighter rule* $P_f = (0.9^{1/E} - 0.1806 \cdot ln(\delta))^E$.

If there is a 40% chance of bad times, a fighter strategy has an evolutionary attractor and ESS. The value measured in the laboratory of a fighter strategy at about E=4.6 is an ESS at around 90% to 95% chance of bad times. It seems to be feasible that the density-dependent fighter strategy as found in *S. berlesei* in the laboratory and when coming fresh out of the wild is an adaptation to short bursts of food with long stretches of low food availability. The fact that value that is close to the actual measured value is

found, shows that the model should achieve some biological reality.

The result of the elasticity analysis can be found in table 4.6. The model was most elastic to juvenile survival. As juveniles are the main deliverer of adults, changes here make the most difference in the overall population dynamics.

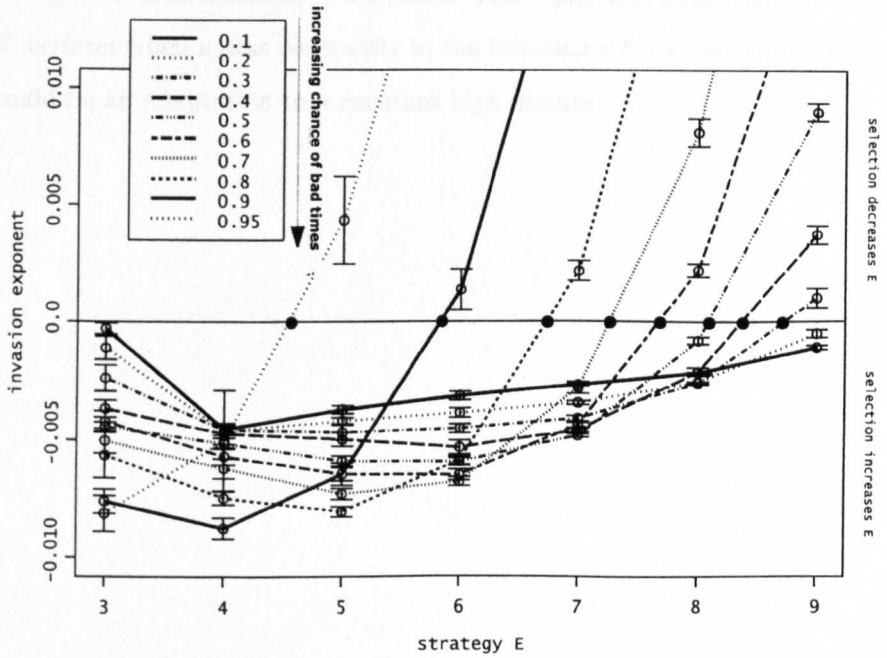

Fig. 4.4: *Result of invasion analysis with 0.975 reduction in strategy ($P_f = (0.9^{1/E} - 0.1806 \cdot ln(\delta))^E$) through different levels of stochasticity. 0.1 = 10 % chance of a bad day occurring ... 0.5 = 50 % of either a good or bad day occurring ... 0.9 = 90% chance of a bad day occurring. At 0.1 a slight decrease in E does not get selected for. Black dots are attractors. The ESS occurs when the invasion exponent=0.*

Changing the fighter rule to $P_f = (0.69^{1/E} - 0.1806 \cdot ln(\delta))^E$ (0.69 strategy) (figure 4.5) shows that the E value of the 0.9 strategy at 90% probability of bad times is nearly reached with 80% probability of bad times in the 0.69 strategy. This means that with the 0.69 strategy fighters develop when there is a higher chance of higher densities (good times). This could be a reaction to higher overall densities of a culture. This value was measured on *S. berlesei* when it was constantly in the laboratory for about two years and could be an adaptation to a constant high density.

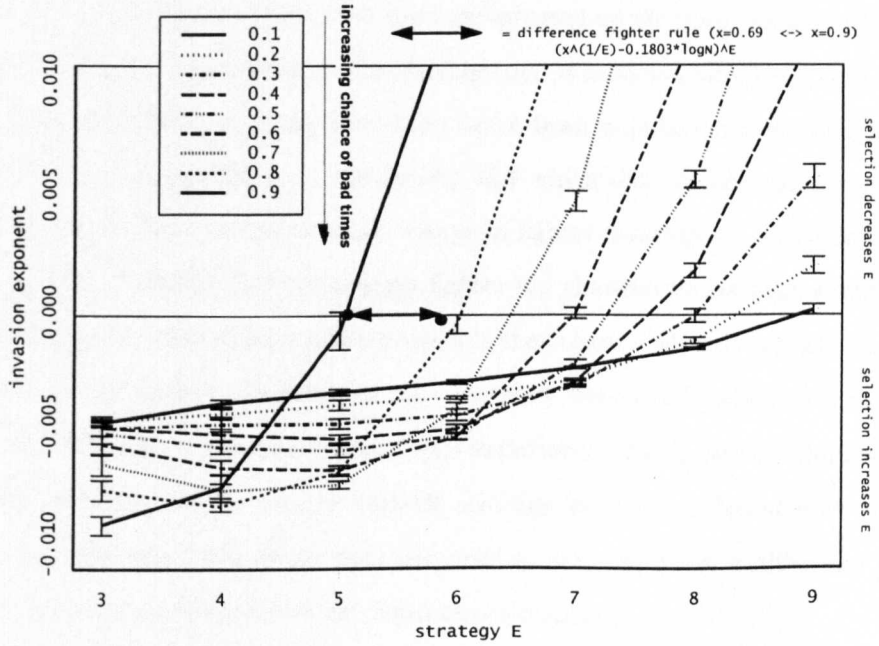

Fig. 4.5: *Result of invasion analysis with 0.975 reduction in strategy* ($P_f = (0.69^{1/E} - 0.1806 \cdot ln(\delta))^E$) *through different levels of stochasticity. 0.1 = 10 % chance of a bad day occurring ... 0.5 = 50 % of either a good or bad day occurring ... 0.9 = 90% chance of a bad day occurring. At 0.1 a slight decrease in E does not get selected for. Black arrow = representative change in ESS rule due to strategy change at 90% chance of low densities. The ESS occurs when the invasion exponent=0.*

4.4 Adding individual variation

4.4.1 Introduction

Previous modelling in this work has concentrated on environmental stochasticity, which concentrated on the stochasticity of food availability. The measured population dynamics have been taken from population level analyses, which do not account for individuality and which did not account for spatial effects. For example a small change in fighter strategy on a population level model implies that the average fighter has changed its strategy slightly. Nevertheless what should really happen is that there is already a slight variation in the fighter development rule, therefore one would have to ask first: What happens to the model if one adds variation to the fighter development rule? Secondly will a slightly variable strategy around a different mean invade? How much alteration does one need to actually make a difference to the population dynamics of the resident population?

4.4.2 Methods

The matrix model has been adapted, using a variable fighter development rule. A Gaussian distribution around the strategy as mean with a variance of 0.10*mean was used. Invasions were done with a $0.975^{*}E$ reduction of the mean using the same individual variation.

4.4.3 Results

The results of the model run with individual variation (see figure 4.6) show that the invasions occur with higher chance of bad times than without individual variation. This indicates that the variance around a mean with Gaussian error works as a buffer. Invasion is only possible with higher probability of bad times. The error bars of the invasion analysis without individual variation (red in figure 4.6) overlap sometimes with the error bars of the model with individual variation. As the error bars overlap sometimes, this could indicate that in these regions the result might not be significantly different.

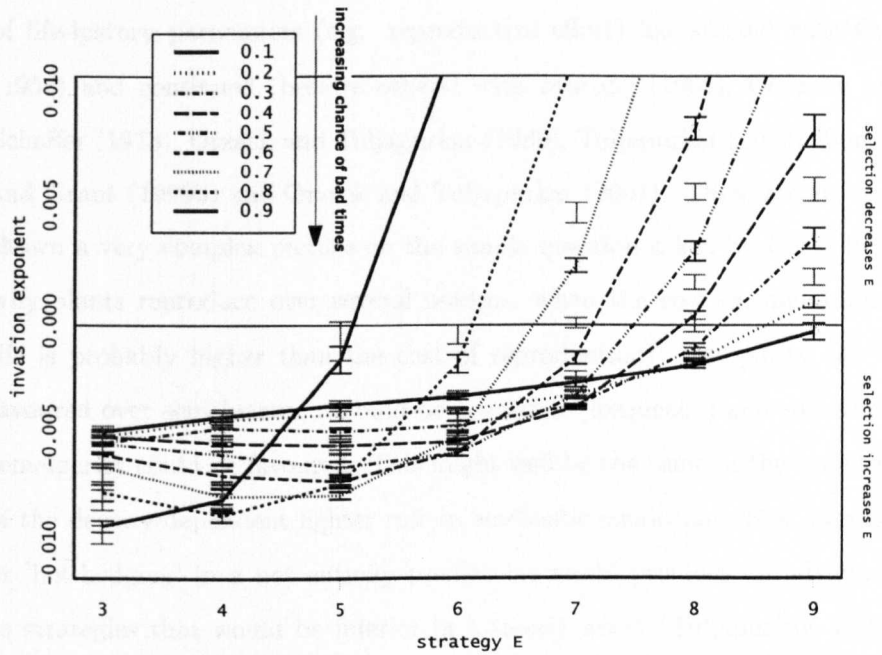

Fig. 4.6: *Result of invasion analysis with 0.975 reduction of mean in strategy* ($P_f = (0.69^{1/E} - 0.1806 \cdot ln(\delta))^E$) *with standard error of* $0.10 \cdot$ *mean through different levels of environmental stochasticity. 0.1 = 10 % chance of a bad day occurring ... 0.5 = 50 % of either a good or bad day occurring ... 0.9 = 90% chance of a bad day occurring. With higher chances of a bad day occurring, there is a higher chance of bad times. At 0.1 a slight decrease in E does not get selected for. Red error bars indicate the shift from the invasion analysis without individual variation. The ESS occurs when the invasion exponent=0.*

4.5 Discussion

The detailed study of effects of environmental stochasticity on the evolution of life-history parameters (e.g. reproductive effort) has started with Cole (1954) and continued (besides others) with Murphy (1968), Charnov and Schaffer (1973), Orzack and Tuljapurkar (1989), Tuljapurkar (1990), Benton and Grant (1999b) and Orzack and Tuljapurkar (2001). These results have shown a very complex picture on the simple question asked by Cole (1954) why plants reproduce over several seasons, when the cost on maintaining life is probably higher than the cost of reproduction? Iteroparity can be favoured over semelparity, it could be a balance (frequency) and sometimes semelparity could be favoured. This might well be the same in the evolution of the density-dependent fighter rule in stochastic conditions. Risk aversion or 'bet-hedging' in a not entirely predictable world provides an advantage to strategies that would be inferior in a steady world (Tuljapurkar, 1990). If this is indeed so, the fighter rule found in stable environments might be not the one which is best in a stochastic environment. Different life stages in *S. berlesei* (eggs, juveniles and adults) respond differently to noise, in that there are significant differences in the way that environmental variation changes the mean, variance, shape of distribution and relationship between environmental and population synchrony (Benton et al., 2002).

The phenotypic plasticity of *S. berlesei* to the environment (in general) is exhibited mainly through condition-dependence and density-dependence (chapter 2). The adaptive advantage of being density-dependent is likely

to be a monopolisation of females and by reducing the population density increasing the output of females in egg mass (see chapter 2 and 3). As growth progresses to the tritonymph stage, the fighter is able to make a relatively safe bet on future generations by detecting the density in its environment [by a chemical cue (Timms et al., 1980)].

The results presented here suggest that the density-dependent morph determination rule is likely to be sensitive to the pattern of fluctuation in the environment, and is likely to adaptively change if the environmental variation changes.

With increasing stochasticity and higher chances of bad track periods, which would through density-dependent food availability reduce the population and therefore generate lower densities more often, an adaptation of the fighter role would be favourable even considering trade-offs.

Ultimately, condition-dependence will limit this development. *S. berlesei*'s morph determination is also condition-dependent, as are other species which exhibit andropolymorphism and a so-called fighter morph [e.g. *J. marmorata* (Borowsky, 1985; Conlan, 1989; Kurdziel and Knowles, 2002) and *O. spec.* (Emlen, 1999)]. Any change of the morph determination rule through often changing densities by stochasticity would therefore likely also apply to these species' andropolymorphisms too. Essentially, through density-dependence in food provisioning, a condition-dependent system will also be limited in the other direction. If densities are too often too low, the fighter morph determination rule cannot be adapted anymore, as simply no food is available anymore to build up extra body mass.

An adaptive fighter rule should be favoured in an unpredictable environment. The data was taken from animals in stable laboratory conditions where the strategy could have adapted to a stable environment (lower probabilities of becoming a fighter in many densities). But when one simulates a highly varied environment, it changes the fighter morph development rule (detected in the laboratory) in a way that resembles the one found in freshly collected animals from the field (higher probabilities of becoming a fighter in many densities). One could conclude from this that *S. berlesei*'s fighter morph has adapted to a highly stochastic environment (e.g. the field).

S. berlesei shows a response to environmental variation in its density-dependent fighter rule. When initially collected in 1996, *S. berlesei* was fed constantly. This resulted in fewer males becoming fighters, when the initial data on fighter morph determination was collected. After the year 2000 the feeding regime was changed to weekly feeding (by Dr. Benton) and the chance of becoming a fighter rose again in very low densities. This could be an effect of different population dynamics affecting the fighter life-history. Therefore, when assessing the propensity of males to become fighters at low densities, there was a noticeable change over time. When the stock cultures were fed daily, fewer males became fighters at a given density. When the stocks were fed intermittently more males became fighters at a given density, perhaps because the population fluctuated more. Experimental evidence of long running cultures suggests that the culturing methodology causes selection on the fighter rule by changing to opportunity for fitness benefits (Wilson, P., 2002 unpub. Honours Project, personal communication Tim Benton).

S. berlesei seems to react also to stochasticity with its additional instar the hypopus which has a higher probability of becoming a fighter (Ballard, 1997). An impact of stochastic population dynamics does therefore exist. This exhibits itself also in the special life-history of *S. berlesei* compared to *Rhizoglyphus echinopus* or *R. robini*. Recently, Radwan (2001) has found the same morph determination strategy as in *S. berlesei* in *R. echinopus*.

R. echinopus does exhibit density-dependent morph determination in comparison to *R. robini*. Both species are bulb mites. The only reported difference between these species, which are sometimes seen as one species, is:

> *"Females lay up to 700 eggs each depending on the host. R. robini tends to form relatively small colonies on narcissus and tulips whereas R. echinopus forms large colonies on a greater range of bulb crops."*
> *(http://ipm.ncsu.edu/AG136/mite2.html)*

It is curious that *R. robini* is said to live in small colonies and yet not have a density-dependent morph determination. The key issue may be both the mean and variance in population sizes. *R. robini* could habitually live at sufficiently small densities that fighters are always present (and so the benefits become frequency-dependent), whereas *S. berlesei* and *R. echinopus* live in colonies which are more highly variable in size, such that at the maxima, the density is sufficiently large to preclude fighter development.

5. ASSESSING THE FIGHTER MORPH RULE BASED ON INDIVIDUALS

Abstract

1.) Using an individual agent based model it is shown that a fighter in high densities has, under a density-dependent fighter rule, less chance to transfer its genetic material to the next generation using simple diploid genetics.
2.) Specifically, the fighter density one is examined where there seems to be no apparent reason to become a fighter (unless immigration is very important).
3.) Some evidence is presented that the density determining rule might not only be decided in the immediate present, but also by gambling on future predicted conditions.
4.) Changing the density-dependent rule to frequency-dependence highlights the interesting property of theoretical coexistence of both morph determinations, frequency-dependence and density-dependence.
5.) If fighter could develop at very high densities, frequency-dependent morph determination would have advantages in reproduction. Nevertheless condition-dependent morph determination might likely inhibit this as condition-dependence is inherently a density-dependent process.

5.1 Introduction

Chapter 4 concentrated largely on population level modelling. A matrix model was parameterised with population-average quantities. In section 4.4 individual variation was introduced and it was demonstrated that this

stochasticity, which could be interpreted as cohort based variance in a strategy, could have an influence on the life-history of the fighter morph of *S. berlesei*. In this chapter some spatial stochasticity and discrete animal sizes will be added within an individual-based model.

Individual-based models differ from most modelling techniques by modelling the individual entities from which a population is built, rather than the average traits of a population. As a result, they typically incorporate more biological detail. This property is especially interesting for this study, due to the investigation of populations with low-density and a distinctive type of individual. Additionally, as the model presented here works in terms of individuals, all arithmetic is integers. Many population-based models, including the matrix model, use continuous/or real numbers (Gillmann and Hails, 1997). Therefore it is possible to have situations where population densities decline to fractions of an individual. To circumvent this, a correction, or rounding factor may be introduced to allow for this "error" (Gillmann and Hails, 1997), an arbitrary extinction threshold incorporated (Gillmann and Hails, 1997) or it is left in as a model property (Gillmann and Hails, 1997). Therefore some individual variation can also be handled in the state-variable models. Grimm (1999) suggest that an inherent problem of individual-based models would be, that they could not deliver answers to general questions of biology because analytical solutions are hard to construct. Individual-based models are thought to be more realistic then state variable models, as they are constructed bottom up as opposed to top down. This means that the system is constructed out of lower level attributes and interactions, where

the dynamics develop "in situ" as compared to a top down approach, where the dynamics are imposed by high level instructions.

Individual-based models are a relatively recent development but are increasingly being used (Batschelder and Williams, 1995; Mooij and Boersma, 1996; Ruxton, 1996; Uchmanski and Grimm, 1996; Wilber and Shapiro, 1997; Beecham and Farnsworth, 1998; Gathmann and Williams, 1998; Santelices, 1999; Keeling and Grenfell, 2000). For example, *Swarm* (Langton et al., 1997), is a tool used to develop individual-based models. It has been used in a number of biological studies, for example to study *Anopheles gambiae* (Carnahan et al., 1997), bacteria (Kreft et al., 1998) and evolution (Strand et al., 2002).

Grimm (1999) suggest that in ten years of individual-based modelling no general answers to biological problems have been found. He nevertheless states that it is possible to learn a lot about the system in question. In most cases the use of IBM's would be forced by pragmatism, as the system in question could not be studied with state-variable models or that the individual properties are important for the scientific question, as it is in this study. Grimm (1999) also states that there is no strong theoretical framework for individual-based models, as it exists with mathematical models. This could simply be due to the lack of challenges the role of the individual in population biology has had.

More recently Strand et al. (2002) finds individual-based models a useful tool in evolutionary ecology, as it can track genetic properties and handle complex interactions better than standard model approaches.

As *S. berlesei* is diploid (Radwan, 1991), the descendants of a single pair of mites (one fighter, one female) would receive an average of 50% of their genes from the fighter. If the mite was one male out of 10, the expectation of the percentage of fighter genes in the population in the future would therefore be under the null hypothesis $E(Pe_f) = \frac{1}{N_f+N_{fe}}$, with $E(Pe_f)$ the percentage of fighter genes, N_f the number of fighters and N_{fe} number of females. Here it is investigated, by simulation, if it is possible that this analytical solution holds when details of the system are modelled explicitly (like space, behaviour). There will be special concentration on the situation where there is a single fighter (and no other males). Where, as the fighter has no competition, the marginal benefits of becoming a fighter are zero. A fighter alone with a female has as much reproductive chance as a non-fighter on its own. Nevertheless single reared males have the highest probability of becoming a fighter (see chapter 2). The advantage of being a fighter here can therefore only lie in fighting off possible immigrants.

Therefore the following is investigated within this theoretical model framework concentrating on the interactions of the individuals:

1.) if the analytical expectation of gene transference in *S. berlesei* can be reliably replicated with the model represented here (i.e. for the fighter strategy to be adaptive, fighters should have more descendants at an arbitrary time in the future than non-fighters under the same conditions).

2.) does the result indicate something more specific about the density-dependence?

3.) does switching the density-dependent rule to frequency-dependence re-

veal possible clues about the underlying principles of morph determination?

5.2 Methods

5.2.1 General methodology

A computer program was written as a framework for the individual-based model (see picture 5.1). Java has the advantage over C of being, from its beginning, designed as an object oriented language, which makes it especially suitable for the individual-based model. To exclude programming language specific influences on the results, it was therefore logical also to decide for Java as the programming language for the matrix model developed in chapter 4. Objects (individuals) were completely encapsulated in their class (a programmatically encapsulated piece of code) and were only able to interact with their environment through specially developed accessor functions. This represents "animal" senses, where an individual is able to interact with its environment through senses, while the environment has an influence on the whole organism. This was modelled as functions regulating the state of the individual. Each object (individual) has therefore states, properties and ways to interact with the environment and is able to receive information about the state of its surroundings. The system was updated at each pixel at a screen resolution of 1024x768 pixels. A fully stochastic simulation run with all repetitions took approximately two weeks (two Dual P3 1 Ghz 512 & 1024 MB Memory), depending on system load. A spatial model was chosen to allow for limitations of movement in high density cultures and

the growing lack of interactions. When there are many objects in the system path blockage is created ("animals" blocking each other from movement; this happens if many animals are close to each other and block each others movement through blocking each others paths). Ideally the system would have to be three-dimensional, but computational restrictions enforced a two-dimensional approach. Given the general "philosophy" of individual-based models as being a bottom up approach, many small parts will result in a bigger picture. This happens in itself by the accumulation of effects of many small rules. This has the trade-off that smaller parts of the system are easily explained but the overall effect is described through the results and not in an overall rule like in top-down models. The accumulation of small events will build this big picture inherently.

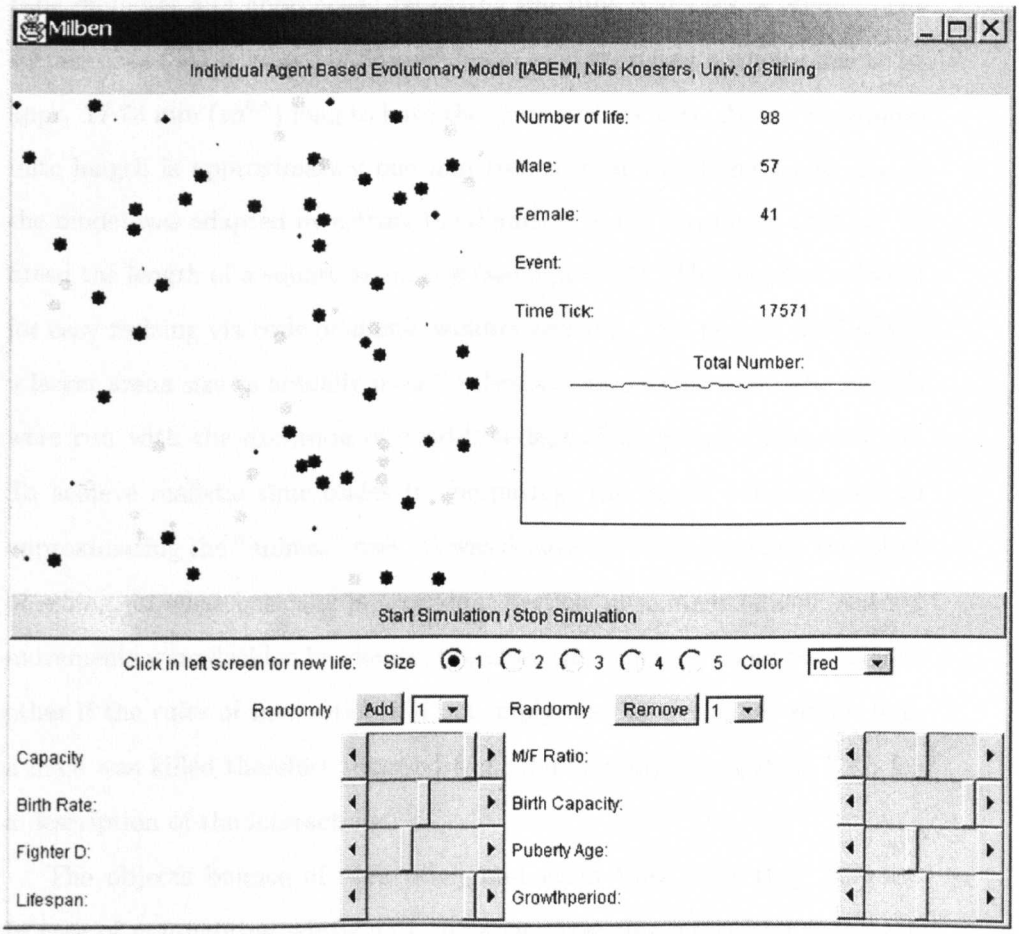

Fig. 5.1: *Java application frame work for an based individual-based model frame work.*

5.2.2 The environment

The arena size was modelled after the tubes used in the experiments. The tube diameter was approached by taking the tube diameter of 20mm. The surface area (sa) is $sa = 314.56mm^2$ ($sa = \pi r^2$) therefore a square has to be appr. 17.73 mm ($sa^{0.5}$) long to have the same surface area. As the maximum mite length is approximately one mm (see section 1.3.1) the arena size of the model was adapted by setting the diameter of the objects so that ca. 18 fitted the length of a square arena size (see figure 5.1). The program allowed for easy resizing via code or simple window resizing. The picture used shows a larger arena size as actually used for demonstration purposes. The models were run with the exclusion of condition-dependent morph determination. To achieve realistic time scales in computing, the model concentrated on approximating the "animal" sizes. It was decided to concentrate on the effect of killing, in what basically is a random particle movement model. Animal movements were decided by chance, therefore the "animals" bounced off each other if the rules of interaction did not imply another course of action (e.g. a male was killed therefore removed from the system) (see section 5.2.5 for a description of the interactions).

The objects bounce of each other and when they meet they interact. In case of non-fighting adults with different sexes this interaction results in procreation. But if males meet and a fighter is involved fighting occurs. When a fighter and a non-fighter meet, or a fighter and a fighter, "animals" can kill each other. When "animals" meet, there are the following rules:

(1) if both animals are males and at least one is a fighter, then a fight occurs, and the non-fighter male dies, or one of the fighters if both males are fighters; (2) if a male and female meet, procreate, (3) otherwise do not interact and just bounce off each other.

All life-history stages like egg dormancy until hatching, quiescence stages between nymphs and adults were ignored. Kills/births happened instantly. This allowed a realistic time frame for the computations. It was assumed that the random effects of interactions would reach asymptotically the amount of interactions in real life. Due to the accumulation of many interaction events it was assumed that these interactions would give a good estimate of the real effects. With this setup enough repetitions could be run to have a stochastic estimate of the results and not just one or two realisations. More complicated stochastic IBM's (Strand et al., 2002) use only two simulations to draw inference on the result. Nevertheless these could occur by chance on such a low sample size.

5.2.3 The individuals

The objects that represent an individual of *S. berlesei* has the following properties taken from good track animals:

1. A static integer defining the size it can grow. An individual has a size property which changes which age. This was based on the fact that *S. berlesei* can grow from ten μm up to around one mm. This reflects *S. berlesei* size properties. Therefore each individual also has an age

integer which keeps track of its age. The size was approximated using the adult maximum size and the environmental size. See section 5.2.2.

2. A boolean switch which sets its state as fighter and a boolean switch setting its state as male or female, drawn from a binomial distribution with $p = 0.5$; *S. berlesei*'s sex ratio has been found to be 1:1 (see section 3.3.2).

3. Age at maturity is set, which changes with environmental conditions. Only after this time interactions between the "animals" lead to offspring. This is based on the accumulated half-lives in chapter 3 and chapter 4.

4. A carrying capacity of 100 adults was set. No reproduction was possible beyond this point. Nevertheless the total population number could temporarily be greater if enough animals were born below carrying capacity. This represents delayed density-dependent effects.

5. A life span is set which determines the "animals" life expectancy excluding fights. The lifespan had a mean of 30 days with a variance of five days, according to observations on good track animals in the laboratory.

6. An integer tracking the past history of an animal is also implemented to track the "animals genetic" line. This determines if offspring stems from a fighter or not. The percentage of fighter related genome is, if

bred with a single female, 50% in the 1st generation. In the 2nd generation it is 25% and in the 3rd generation it is 12.5%. If a female with 50% fighter gene material is "bred" with another fighter in the second generation, this "animal" with 50% original fighter gene percentage would have $\frac{50\%+12.5\%}{2} = 31.25$ percentage of original fighter genes.

7. Animal speed differed between the four instars used omitting eggs. A larva had approximately a fourth of the speed of an adult. This means that a larvae would cross the tube length in about 3.3 minutes while an adult could do this in about 40 sec. The model therefore implemented a relative speed difference between larvae, protonymphs, tritonymphs and adults, by making an adult about five times faster than a larvae ($\frac{198s}{40s} = 4.95$). The speeds of proto- and tritonymphs were fixed at equidistant (linear) differences between a larva's and an adult's speed. Individual life-history characters were calculated as in chapter 4, but using the standard error for bandwidth of individual variation.

5.2.4 The fighters

Males become fighters after the rule established in formula 5.1 and as also found in chapter 4. If a male "decides" to become a fighter, he develops faster than those becoming non-fighters. This increase in speed (maturing in 86% of the time) was based on (Radwan, 1995) and personal observation.

$$P_f = (0.9^{1/E} - 0.1806 \cdot ln(\delta))^E \tag{5.1}$$

The probabilities for fighter aggression in case of interactions were used according to section 2.1.1. In frequency-dependent simulations the fighters were determined by set frequencies and the density-dependent rule was removed.

5.2.5 The interactions

At each interaction (the coordinate overlapping of two agents / "animals" / individuals) the properties of the "animals" were assessed.

- When a fighter and a non-fighter meet the non-fighter's probability of survival was assessed using the data from section 2.1.1 divided by the time span the data was taken, assuming a linear relationship.

- When a fighter and a fighter meet the fighter's probability of survival was assessed using the data from section 2.1.1. Since the probabilities where assessed as the probabilities of one fighter surviving a rule for this interaction had to be found. Observation in the lab shows that in most cases the killing is done by one male mounting the other on the back and killing it. Therefore one animal was selected as the aggressor. With a probability of 50% the outcome was drawn from a binomial (1,0.5) distribution. If the outcome was zero the aggressor died, if one the defender died.

- When a male and a female meet the number of off-spring was assessed

using data from section 3.3.3 and rules established in section 4.2.11 so that fecundity varied with food amount available.

- When non-fighter met a non-fighter or a juvenile met with any adult, no action was taken.
- Eggs were omitted from the model as they do not interact.

5.2.6 Simulations

Estimating the advantage of being a fighter at different densities applying a density-dependent fighter rule

Fourteen different starting combinations of fighters and non-fighters were run 500 times, using the setup in table 5.1 until the model reached approximately steady state. One male was chosen (when fighters were in the system) and assigned a value of his "genetic" material, 100 percent. At each "mating" interaction, the sum of the "genetic" material of both "animals" (male and female) was added and divided through two, assuming diploid genetics and that 50 percent of each "parents" genetics end up in their "offspring". At the end of the simulation the percentage of genetic material stemming from that original male was logged for all animals and later analysed with a standard statistics program, to determine if it transferred more genes than an average male into future generations.

n_{sim}	f	nf	fe	n_{sim}	f	nf	fe
1	0	1	1	2	1	0	1
3	1	1	2	4	0	6	6
5	6	0	6	6	3	3	6
7	0	10	10	8	10	0	10
9	5	5	10	10	20	0	20
11	1	19	20	12	1	49	50
13	50	0	50	14	0	50	50

Tab. 5.1: *Fighter non-fighter starting combinations of simulations with density-dependent morph determination rule. f = fighter, nf = non-fighter, fe = females. n_{sim}=label of simulation.*

Estimating the stability of the model

To test the elasticity of the model percentage differences to the model parameters were randomly tested. An individual-based elasticity analysis was conducted using 95% of the mean values of the traits in question. In the case of area size, 120% of the original area size was used to see if widening the area would change the result, as test runs could not determine a change in model outcome with a change to 95% of the area size. A greater area size reduces the probability of "animals" meeting and should therefore generally reduce the probability of animals interacting with each other. This should have an adverse effect on the advantage of fighters killing other males. Therefore widening the area should lower the advantages of being a fighter at previous good densities (densities where the fighter should have a high advantage as tested in previous chapters). Due to the extensive time these simulation have to run (appr. two weeks), model parameters (variables) were chosen to represent the more extreme densities, fighter—non-fighter combinations

and five defining variables of the model. Fighter kill probability was chosen as it is one of the main determining components in a fighting system. Area size was chosen as it was approximated. The interval between life stages, maturation age and overall life time were chosen as they are important for the density of one male, where a fighter's advantage of being a fighter depends on killing next generation males. This of course is only possible if good food conditions prevail and the fighter actually interacts with the next cohort. By now, no advantage can be seen to become a fighter alone, with no other males around, when he matures very slowly and dies before the next cohort arrives. If the chance of generation overlap is lowered, the chance of encountering next generation males is lowered, and the system should be sensitive to this. Theoretically, if there was no overlap of generations at all, there should be no advantage of being born a fighter at this density and the extra investment of thickened legs should be useless, if no immigration takes place.

Estimating the advantage of being a fighter with a frequency-dependent fighter rule

Ten different starting combinations of fighters and non-fighters (density 100, equal numbers of males and females) were run 500 times, starting the population with ten different frequencies (0.1-1.0 in steps of 0.1) until the model reached approximately steady state. Unlike in section 5.2.6 there was no density-dependent fighter rule, but frequency-dependent morph determination. Therefore approximately as many fighters (drawn at each birth from a

uniform distribution) were born as the frequency chosen in the current run. One fighter was chosen and assigned a value of his "genetic" material, 100 percent.

5.3 Results

5.3.1 Estimating the advantage of being a fighter at different densities using a density-dependent fighter rule

As expected one animal (one non-fighter) manages, in a starting population with a non-related female, to transfer about 50% of its genetic material into future generations (figure 5.2 #1). One fighter with one female manages more than 50% as it can kill also males of the next generation (figure 5.2 #2). With one fighter, one non-fighter and two females, the fighter's ability to eliminate competition enables it to nearly reach the levels of genetic transference into future generations of one non-fighter, but with more extreme values. This can be interpreted as that a male can have potentially a higher pay-off when he gambles on being a fighter (figure 5.2 #3). In short, being a fighter pays off at this level. When a population is started with six non-fighters and six females the long running expectation is 8.33 % of "genetic" material to be transferred from one individual into the future generations (figure 5.2 #4). This is indeed the case in this simulation. When six fighters are together the mean transference sinks dramatically although a fighter has, potentially, the chance

that he can transfer more of its genetic material into future generations (figure 5.2 #5). If the odds are 50:50 with three fighters and three non-fighters, one fighter has, at the density of 12 "animals", the chance to transfer more of its "genetic" material into future generations (figure 5.2 #6), as he kills other males, but at a risk to be killed himself.

At a density of 20 "animals" the long running "genetic" transference expectation is 5% (figure 5.2 #7). Again the fighter has potentially the chance to increase its ability to father a greater proportion of descendants, nevertheless the chances are becoming lower (figure 5.2 #7 8 9). At even higher densities, one fighter with many non-fighters, the fighter typically increases its proportion of descendants compared to non-fighters (figure 5.2 #11,12). Nevertheless, when it would be beneficial to be one fighter all "animals" might be deciding on this strategy, which would decrease one "animals" chance of becoming the father of many generations(figure 5.2 #10, 13), therefore one would expect that it is not "worth" becoming a fighter at these densities, due to frequency-dependence.

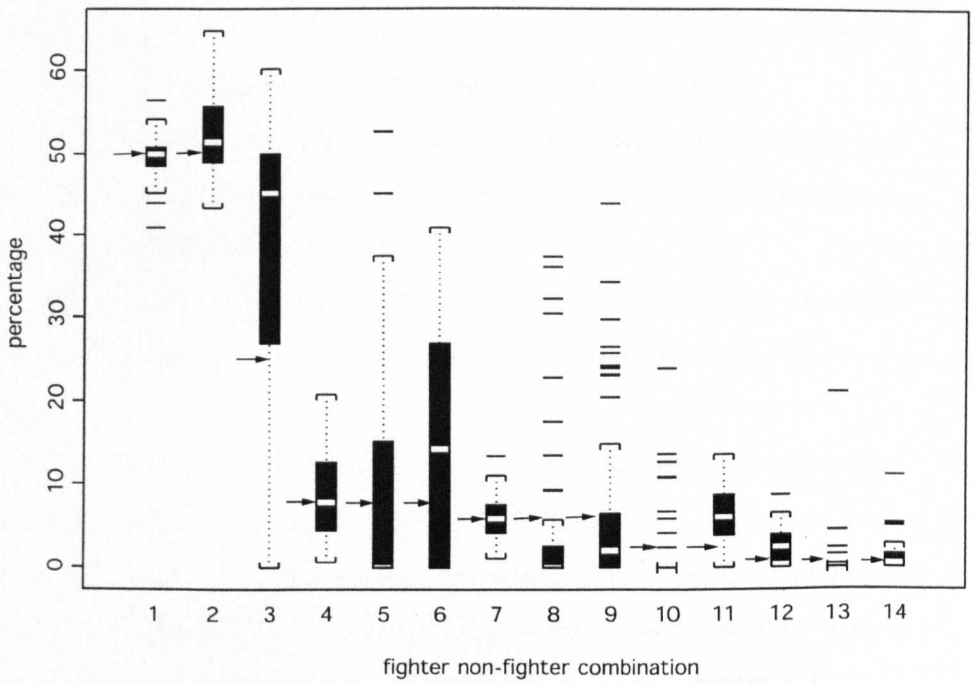

Fig. 5.2: *Box plots of simulations (n=500 per combination) of the percentage contribution to the population of a focal male where several combinations of fighters and non-fighters were simulated (female density equaled male density). The percentage of "genetic" material of a focal "animal", assuming diploidy, and random mating w/o sperm precedence vs density combination is portrayed, after population reached steady state. Combinations: 1=0 fighter 1 non-fighter, 2=1 fighter 0 non-fighters, 3=1 fighter 1 non-fighter, 4=0 fighter 6 non-fighter, 5=6 fighter 0 non-fighter, 6=3 fighter 3 non-fighter, 7=0 fighter 10 non-fighter, 8=10 fighter 0 non-fighter, 9=5 fighter 5 non-fighter, 10=20 fighter 0 non-fighter, 11=1 fighter 19 non-fighter, 12=1 fighter 49 non-fighter, 13=50 fighter 0 non-fighter, 14=0 fighter 50 non-fighter. Black arrows point at expected mean w/o fighter involvement. Fighters do well at low densities at low frequencies, except at density one, where the difference depends in this closed system only on killing their own offspring and mating with their daughters when there is a generation overlap. Killing of their own offspring and mating with their daughters is a biologically observable reality in S. berlesei.*

Simply looking at the fighting aspect of this model clarifies why fighters should have only an advantage in lower densities. The individual-based elasticities (figure 5.3) show that the above result is relatively stable, in higher densities. The overall answer of the original run is not altered.

The only interesting change is that all calculated elasticities show that the combination of one fighter with no competing male gives the same chance as being a non-fighter on its own.

Therefore for a fighter to be effective at density one male one female, he has to meet enough next generation males to gain an advantage of being a fighter.

The advantage of being a fighter in a newly selected patch is density-dependent (number of other males per area on patch). If the area is too large, the chance of two males meeting is lowered, reducing the possibility of monopolising females. So if an increase in area size makes it less likely to meet next generation males the fighter's advantage of being a fighter is lowered. One could label this as a delayed density-dependent morph advantage (the advantage of being a fighter is delayed until the next cohort arrives). So the morph determination rule at a density of one is not only determined by present densities but by the selective advantage created by the ability to kill future generations. The effect will be much stronger in real life, as fighters can live up to 60 days and possibly have an influence on up to four or five generations ahead. Depending on the density-dependence then, (more animals are less good for the fighter morph) the influence will vary, but will still increase a fighter's probability of transferring more of its genome into

the future.

Nevertheless, when the immigration of other males is of importance, it makes sense to be a fighter at density one. The fighter protects its patch. Here is therefore a split cost benefit scenario. At density one the advantages of being a fighter is only defined by the probability of encountering new immigrants and meeting future generations. But if density increases (due to many eggs hatching at the same time with a fighter) the fighter's advantages are more and more influenced by the mites already around him. This can be seen as information that is available to him, in contrast to taking a risk. Theoretically, ten new fighters could immigrate into a patch where a fighter is alone, but here the fighter takes a gamble, while with already existing mites while he is growing up, the future becomes much more predictable.

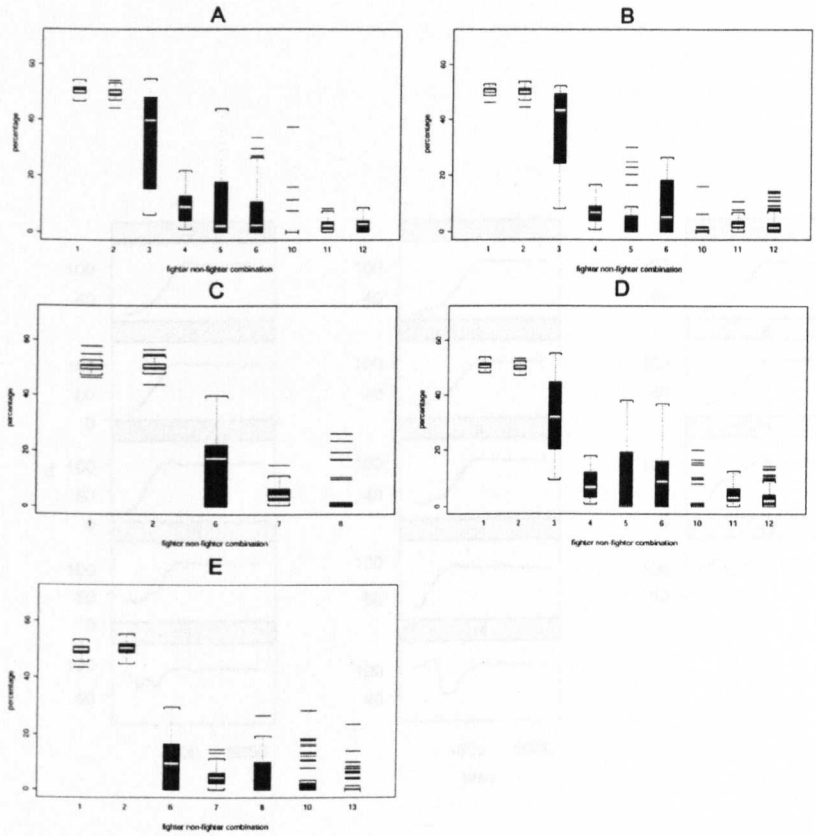

Fig. 5.3: *Box plots of individual-based elasticity simulations (n=500 per combination) where several combinations of fighters and non-fighters were simulated (female density equals male density). A) area size 120%, B) fighter kill probability 95%, C) interval between life stages 95%, D) life time 95%, E) age at maturity 95%. The expected percentage of genetic material of one "animal", assuming diploidy, and random mating w/o sperm precedence vs density combination is portrayed, after population reached steady state. 1=0 fighter 1 non-fighter, 2=1 fighter 0 non-fighters, 3=1 fighter 1 non-fighter, 4=0 fighter 6 non-fighter, 5=6 fighter 0 non-fighter, 6=3 fighter 3 non-fighter, 7=0 fighter 10 non-fighter, 8=10 fighter 0 non-fighter, 9=5 fighter 5 non-fighter, 10=20 fighter 0 non-fighter, 11=1 fighter 19 non-fighter, 12=1 fighter 49 non-fighter, 13=50 fighter 0 non-fighter, 14=0 fighter 50 non-fighter.*

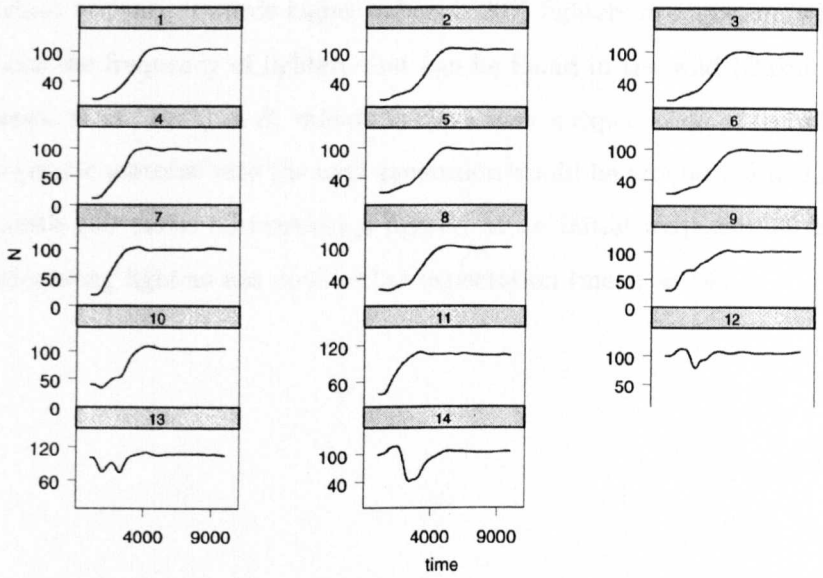

Fig. 5.4: *Example time-series of simulations (n=1 per combination) of adult densities where several combinations of fighters and non-fighters were simulated (female density equals male density). Adult density, assuming diploidy, and random mating w/o sperm precedence vs density combination is portrayed, after population reached steady state.1=0 fighter 1 non-fighter, 2=1 fighter 0 non-fighters, 3=1 fighter 1 non-fighter, 4=0 fighter 6 non-fighter, 5=6 fighter 0 non-fighter, 6=3 fighter 3 non-fighter, 7=0 fighter 10 non-fighter, 8=10 fighter 0 non-fighter, 9=5 fighter 5 non-fighter, 10=20 fighter 0 non-fighter, 11=1 fighter 19 non-fighter, 12=1 fighter 49 non-fighter, 13=50 fighter 0 non-fighter, 14=0 fighter 50 non-fighter.*

5.3.2 Estimating the advantage of being a fighter with a frequency-dependent fighter rule

As can be seen in figure 5.5 the strategy with the highest median and the highest variance towards higher values is 20% fighters in a system, which is about the frequency of fighters that can be found in the wild (Baker, 1983; Gerson et al., 1983) in *R. robini*. While a male's expectation of transferring its genetic material into the next generation would be around 1% within 100 animals (50 males 50 females) a fighter, at an initial frequency of 20% of males being fighters has double that expectation (mean $\approx$ 2%).

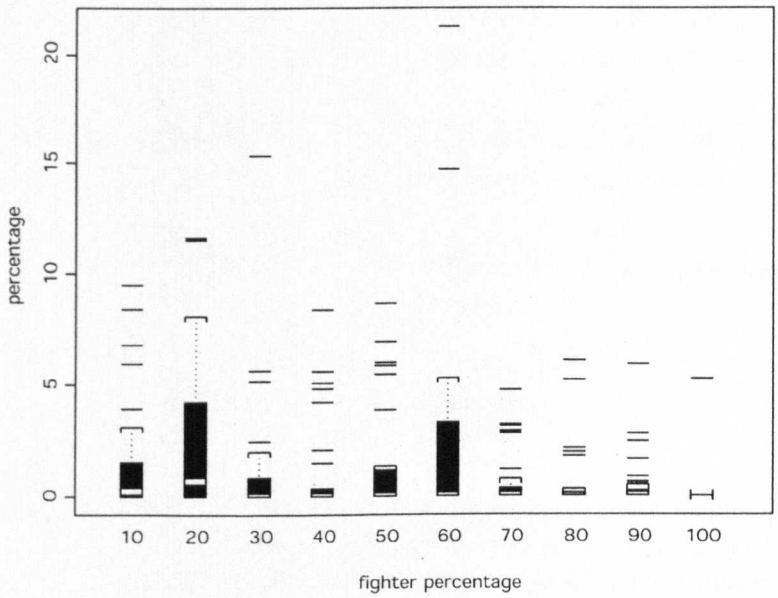

Fig. 5.5: *Box plots of simulations (n=500 per combination) where several combinations of fighters and non-fighters were simulated (female density equaled male density; with a total density of 100) and a frequency-dependent fighter rule. 10 - 100 percentage of fighters in the system. The percentage of "genetic" material of a focal "animal", assuming diploidy, and random mating w/o sperm precedence vs density combination is portrayed, after population reached steady state.*

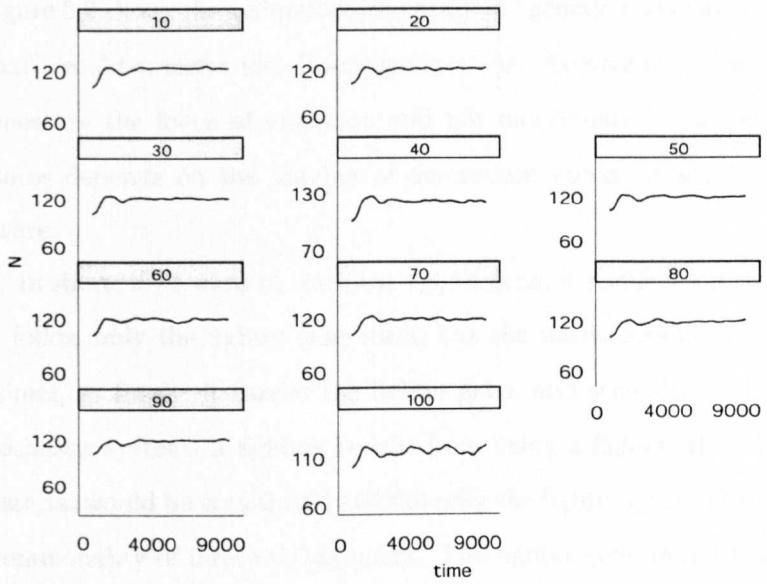

Fig. 5.6: *Example time series of simulations (n=1 per combination) at density 100 where different frequencies of fighters and non-fighters were simulated (female density equaled male density). 10 - 100 percentage of fighters in the system. Time series starts at 0.*

5.4 Discussion

Figure 5.2 shows the estimated percentage of "genetic material" that one "animal" would transfer into future generations. According to Dawkins (1989) genes are the focus of evolution and not individuals or species. A gene's fitness depends on the number of descendant copies at some point in the future.

In short, if we want to track the fighter gene, it seems to be not important to follow only the fighter gene itself, but the whole genetic material of the animal, as firstly, it carries the fighter gene, and secondly, in long running stochastic systems if fighters benefit from being a fighter, the whole genetic material would be transferred and not only the fighter gene. This is Dawkins' "commonality of interest" argument. The fighter gene would therefore be a carrier for other genes. This means that the fighter gene, or the ability to dominate other males and to transfer genes would benefit all genes and not only the fighter gene.

When conditions are stochastic, as it happens with *S. berlesei* and there are, through migration to other food sources, extreme density differences and fast population growth a fighter can achieve a much higher genetic contribution to future population size, if he is able to adapt to a changing situation (see figure 5.2).

Although a fighter certainly has a trade-off occurring through not being to mate as often, when he is fighting more than mating in high densities (Ballard, 1997), the obvious trade-off is also the simple competition by num-

bers. The lower fecundity by females in higher densities (see section 3.3.3 and table 3.2) makes each males mating worth less, in terms of fecundity.

Additionally the individual-based model shows that it is possibly important to have overlapping generations for the fighter rule to work as it is found in the laboratory. In non-overlapping generations the fighter would have no advantage in being a fighter, when paired with one female (at density one fighter paired with one female) as it's chances of transferring his genetical material into the next generations would be equal to that of a non-fighter at this density (50%). A fighter has therefore no advantage of being a fighter as there are no other animals to fight with, unless immigration brings them. But this makes the prediction of future densities more insecure. Therefore the main gamble the fighter takes at this point is that few males immigrate. If he does not encounter any immigrants, he nevertheless might still profit from being able to kill his own offspring.

Local mate competition (female bias arises because it reduces competition among brothers for mates, and because it increases the number of mates for each of the female's sons) (Hamilton, 1967; Flanagan et al., 1998) cannot be observed, as *S. berlesei* has a 1:1 sex ratio. The detailed influence of local mate competition in delayed density-dependence would have to be researched in more detail. But as *S. berlesei* is a diploid species opposed to the haplodiploid parasitic wasps [which makes sex control easier (Flanagan et al., 1998)] it is unlikely that a female sex bias can be found.

It has indeed been statistically tested as not diverting significantly form 1:1 (see section 3.3.2). However, a reduction in LMC could come about by

fathers killing their sons (reducing both, competition for mates and competition for food) if there was no genetic mechanism for sex ratio adjustment. Or, killing one's own offspring (which fighters actually practice) could be simply maladaptive, and represent the a situation unlikely to arise in the field (perhaps because there will be sufficient mixing between patches that the likelihood of encountering your male offspring is sufficiently unusual not to be selected against).

Therefore there seems to be no great advantage to being a fighter at density one paired with one or several females in a closed system. In an open system with immigrating males, it is likely advantageous to be a fighter if other males invade into a fighters patch and he has actually something to fight with. Immigration events should therefore be an essential part in *S. berlesei* morph determination rule.

Within one density fewer fighters do better than more fighters (see figure 5.2 5 and 6; 8 and 9; 10 and 1; 12 and 13) and even in very high densities (see figure 5.5) a fighter could potentially gamble on achieving a higher fatherhood, if there would be a 20% frequency-dependence rule. The models predict that fighter morph determination should be frequency-dependent in stable conditions. This matches empirical findings, where one fighter together with four non-fighters always does better than five fighters together. Each density seems to have an underlying frequency-dependence. Therefore, in a stable environment selection on fighters is to respond to the frequency of fighters rather than population density.

Fighters in *S. berlesei* in higher densities have until now only been found

in one uncontrolled laboratory experiment, with high structural stochasticity (mites in vermacolite) and predator presence (conducted by Dr Benton). This could nevertheless been seen as an accumulation of many low density populations in close proximity. A fighter can, through random patch isolation through the filling structure (in this case vermacolite), defend his patch and therefore this would approximate low density conditions. They might need more detailed population dynamics or might be unimportant compared to the advantages gained by a density-dependent morph determination rule or might not be evolutionary stable. The most likely reason is, that fighters at high density levels as experienced by *S. berlesei* would not develop through condition-dependence as there would simply not be enough food available to be invested in the necessary extra leg and body mass to allow a fighter to be at advantage in a fight.

Therefore in reality, frequency-dependent morph determination could not exist at high densities even if a frequency-dependent morph determination rule would give an advantage at certain animal combinations (figure 5.5). There will simply be not enough food to build up the physical traits to constitute a different phenotype. This will be discussed in more detail in the next chapter.

For andropolymorphism (and polymorphism in general) one can therefore conclude that the cost and benefit structure of a frequency-dependent morph determination rule as can be found in *R. robini* (Radwan, 1996; Radwan and Siva-Jothy, 1996), *O. spec.* (Emlen, 1999), Salmon (Garcia-Vazquez et al., 2002) and other species (Gross, 1996) depends on a more complicated inter-

play of population dynamics and interactions between the morphs. While the simple battle by numbers and the frequency of their occurrence seems to be a sufficient explanation for density-dependent morph determination, this does not seem to be the case for frequency-dependent morph determination, as indicated by game theory (negative frequency-dependence = lower numbers do well), if we see fitness as a game for maximising once own genetic material. Nevertheless it could be possible that if the population dynamics are cyclic that both strategies could exist as recently demonstrated in side-blotched orange lizards (Sinervo et al., 2000).

6. CONCLUSION

The data and the models reinforce the theme present in many life-history studies: vital rates are sensitive to variation in the environmental conditions that an organism experiences (Stearns, 1992). While it is probably expected that plasticity will occur at multiple points in an organisms life-history, detailed assessments of patterns and discrete polymorphism linked to experimental variation in environmental characteristics, has rarely been established. Plasticity is generally accepted as a life-history strategy for variable environments. Evidence that a discrete polymorphism can respond to environmental variation is presented in this thesis. The expression of every trait examined in the mites' life-history was sensitive to the immediate and recent historical densities, food amounts and starvation periods experienced by the organisms (chapter 3) (Beckerman et al., 2002, 2003).

However it is not only the "general" life-history of the mites but also the discrete phenotype, which has to exist in a varying environment. With limited time, energy, nutrients available for growth and reproduction, the *S. berlesei* fighter morph has to trade-off when maturation and development is necessary to reach the age of first reproduction. A fighter morph does this by developing earlier than other males (Radwan, 1995), and he mates and

feeds continuously (see section 1.3.5 and chapter 2) and reproduces in his life-time iteropar. Fighters can live very long (Radwan, 1995).

Fighters in *S. berlesei* develop at lower densities in high food conditions. If they are alone with no other fighters around they have the possibility of monopolising all females and can therefore become the fathers of all future generations. There are two extreme possibilities in this scenario, kill all other males before they become adults, or kill all adult males.

Fighters develop faster between the last two moults (Radwan, 1995), so they theoretically have the opportunity to kill non-fighter tritonymphs as they emerge later from the dormant tritonymph stage. However killing of tritonymphs was rarely observed, as it should be maladaptive to kill ones own offspring, or sex recognition is not possible for the fighter at this stage.

Matrix models do not cope well with individual variation, especially in plastic systems like the one presented in this thesis. While the matrix model investigated the effect of changes in stochasticity on the fighter morph determination rule, the individual based model introduced individual variation and stochastic interactions. As a result the IBM confirmed the benefits of a density-dependent fighter morph determination for *S. berlesei*. Fighters in lower density make a greater genetic contribution to the future genetic makeup of a *S. berlesei* population. The IBM also hinted at the possible presence of an underlying frequency-dependent fighter rule. Given the knowledge that also a condition-dependent morph determination rule exists in *S. berlesei* the results of the matrix model and the individual-based model can be synthesised into the following hypothesis.

The phenotypic plasticity of *S. berlesei* to the environment exhibits through condition-dependence, density-dependence and frequency-dependence. In the mite system, males develop into fighters at low population densities, when typically food is common. However the inverse link between resources and population density is not always the case (as, for example, following exhaustion of resources in a patch the density falls but resources are not freed up). Given the necessity to devote extra resources to weapons if a male develops into a fighter, it is hypothesised that fighter development is likely to be condition-dependent as well as density-dependent. Such a situation can also be found in the amphipod *J. falcata* (Kurdziel and Knowles, 2002).

In addition to condition-dependence and density-dependence, frequency-dependence may play a role in determining in a polymorphic system. Frequency-dependence occurs where the costs and benefits in a system vary with the frequency of the strategy. For a mixed ESS to occur the fitnesses of the morphs need to be equal [e.g. lekking ruffs (Lank et al., 1995)].

In a dynamic system with density-dependence acting, the term frequency-dependence has some ambiguity (Heino et al., 1998). Nonetheless polymorphism in the mite fighter/non-fighter system is likely to involve some frequency-dependence as the costs involved in defending a harem of females are "proportional" to the number of fighters. In the related mite genus *Rhizoglyphus* a species is recorded as having a fixed frequency of fighter morphs in the population (presumably maintained by frequency-dependent selection) (Radwan, 1995), whereas another species is reported to have density-dependent morph determination as in *S. berlesei* (Radwan, 2001).

It is even possible that, for example, condition-dependence is a contributing mechanism by which density-dependent morph determination is maintained as males at high population densities are likely to have reduced per-capita resources available. Even when a male acquires stochastically enough food in a high density low food situation, the law of large numbers predicts that this will not be the case for all males. Frequency-dependence can therefore more likely be expected in animals living in a predictable, non-stochastic environment as *R. robini*, which is a pest of grains, onion, garlic and leek in the field and of bulbs (Gerson et al., 1983; Chen, 1990), and which has a frequency-dependent morph determination although it is very similar to *S. berlesei* (Radwan and Klimas, 2001). Nevertheless if the mean expected density of a population rises, like in *R. echinopus*, the morph determination seems to be density-dependent (Radwan, 2001).

Therefore the following scenario of condition-dependence, population dynamics and morph determination for the three mite species out of the Acaridae which exhibit the same polymorphism can be fixed as described in table 6.1.

species	extra leg mass	morph-determ.	density	environment
S. berlesei	yes	density dep.	often high	stochastic
R. robini	yes	frequency dep.	low	stable
R. echinopus	yes	density dep.	high	stable

Tab. 6.1: *Representation of the condition-dependent morph determination, type of morph determination, anecdotal population density and type of environment.*

Condition-dependence occurs if the fitness costs and benefits depend on resources. If density is linked to food resources pure frequency-dependent morph determination, in a phenotype which has to build up extra body mass, should only exist if the food related density-dependence is very weak or the costs are very low. Species that experience very high density-dependence on the trait of physical body mass alteration should only be able to have a frequency-dependent morph determination if they exist most of the time substantially below carrying capacity.

A high amplitude in density levels coupled with condition-dependent morph determination should therefore push the *S. berlesei* system into density-dependent morph determination. If we follow this theoretical thought one could imagine that also the *R. echinopus* system, which also operates in higher densities, has to succumb to the pressures of density-dependence. Furthermore this model system would suggest that the fighter morph determination as found in *R. robini* can stay in the frequency-dependent as it operates in medium densities, on very nutritious feeding areas (bulbs). Ample food is available, so frequency-dependent morph determination is possible.

Other species with body related polymorphism that reduce their body size can therefore "afford" to have a frequency-dependent morph determination. Sneakers in Salmon (Garcia-Vazquez et al., 2002) are small and therefore the physical costs of becoming this morph are low.

If one takes the example of lizards showing r and K selection depending on polymorphism (Sinervo et al., 2000), one could imagine this to be a reaction to the essential influence of condition-dependence. Heino et al. (1998)

also points out that pure frequency-dependence is essentially not possible in dynamic systems. Wakano et al. (2002) finds in a computer model changing frequencies in cannibalistic amphibians, according to the hypotheses that population cycles could produce polymorphic strategies. Therefore also the population dynamics of animals have an essential influence on polymorphism and the kind of morph determination.

One could therefore conclude that through condition-dependence on body size there is no pure frequency-dependent, density-dependent or condition-dependent morph determination, but only one polymorphism (thickened third leg) ruled by a continuous covariate of the trait of density-dependent body size alteration. This would explain, why in relatively closely related species, such as *R. robini*, *S. berlesei* and *R. echinopus* the same expression of two distinct morphs exists, with apparently different morph determinations. Which morph determination is possible could simply depend on variations of food allocation.

If the process of splitting the acarid mite polymorphism over several species involved sympatric or allopatric speciation is yet to be tested. It seems to be possible that the ancestor(s) of the polymorphism, found themselves suddenly through phoresy in a strongly density-dependent system and had to adapt, or vice versa, that a polymorphism evolved under density-dependence, suddenly found the bulb habitat. Capua and Gerson (1983) classified *R. robini* as an ancient soil mite, therefore the process of *R. robini* and *R. robini* becoming "bulb mites", as their common name suggests could be a fairly recent event.

Therefore one could conclude generally that even discrete polymorphism concentrated on one sex can be a very dynamic inter-species system and might be an explanation of differences of polymorphism in closely related species, like the whole *Jassa* genus (Kurdziel and Knowles, 2002), the lizard complex (Hews et al., 1997) or the loss of polymorphism as between *Salmo salar* and *Salmo trutta*. Especially interesting should be the study of polymorphism in the Salmon gender where hybridisation occurs (Garcia-Vazquez et al., 2002) and polymorphism is lost, if the sneaking polymorphism is an alternative strategy. Adaptive radiation of polymorphism in several systems as a marker, might therefore provide more insight into speciation itself, be it allopatric, sympatric or parapatric.

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APPENDIX

A. ADDITIONAL STATISTICAL ANALYSIS

As extra information the following statistical analysis is given below and has been taken from earlier drafts of Beckerman et al. (2002, 2003).

A.1 Methods - survival data

Because survival analysis sample sizes are based on the number of events that occur, and our sample sizes were extremely large (33,000 for egg [n=2 days of sampling] and 95,000 [n=4 days of sampling] for juvenile analyses) we developed a protocol to maintain some conservatism in our interpretation of significance levels. Our protocol was as follows:

1) Display the data using Kaplan-Meier estimates of hatching, survival or recruitment stratified on single treatments. Each graph shows patterns of survival stratified by a single factor in our experiment, averaging over the other treatments and does not represent a controlled statistical analysis.

2) Choose an initial distribution using a probability plot; in all survival analyses, the data was compared to seven different distributions.

3) Specify a model that contains up to 4-way interactions (maximum number of interactions for adults) or 3-way interactions (maximum number possible for the juveniles) and use the change in explained deviance caused by moving to a less specified model to judge the relevance. If by moving from a 4-way to a 3-way, the change in deviance was significant but less than or equal to three percent, we assumed that the higher interactions while statistically real (which often they were given our sample sizes), they were likely to be biologically insignificant [(Crawley, 1993), K. Wilson personal communication]. This was repeated for the 3- to 2-way and the 2- to 1-way models generating a conservative model.

4) Once the order of the model was chosen, AIC values were used to iteratively reduce the model. Because an AIC based algorithm [stepAIC function in the MASS library for S-PLUS 2000 (Venables and Ripley, 1999)] chooses a best predictive model, the result of the AIC can contain terms with p-values >0.05. The AIC result was thus further reduced manually based on p-values, to remove insignificant terms, if they existed, generating the minimum adequate model (Crawley, 1993).

5) Use distribution diagnostics to check assumptions of the model distributions (e.g. linearity of survival function on a log-log plot for Weibull distribution) and assess fits (e.g. time at which 10,50 90% events happened) vs raw data.

A.2 Methods - non-survival data

All non-survival data was analysed with a generalised linear model employing a similar strategy beginning with model reduction (AIC and or manual reduction) to generate the minimum adequate model. Data transformations (e.g. log-transformations) were employed when needed and a Gaussian error distribution specified. The percentage of mites recruiting was analysed using a binomial generalised linear model (binomial error distribution), with a dependent variable coded as a dual response of success (recruits) and failure (maximum juvenile density recruits).

Given the high number of interactions in our experiments and the subsequent possibility of inflated significance, P values in the final models for both survival and generalised linear models were further validated through bootstrapping to estimate 95% confidence intervals around the regression coefficient estimates. If the 95% CI included zero, then the interaction was dropped from the model and any adjustment of previously significant terms reassessed.

All analyses were performed with S-PLUS 2000. Certain functions and techniques were used from the MASS library (Venables and Ripley, 1999), the HMISC library (Harrell, 2000) and the DESIGN library (Harrell, 2000) for S-PLUS.

	Df	F	Pr(F)
days laying	1	53.45728	1E-08
delay	1	40.55874	1.4E-07
food amount	1	5.18679	0.028
rearing conditions:adult density	1	9.32625	0.004
Residuals	40		

Tab. A.1: *The minimum adequate model for the effects of density, delay and food on lifetime reproductive success. Descriptions of the factors used in the models are in the Methods section. Days laying is a covariate controlling for differences in egg numbers caused by certain females being alive for longer.*

	Df	Deviance	Df	Resid. Deviance	$Pr(\chi^2)$
NULL			2	8667.367	
egg batch	-1	3679.572	3	4987.794	< 0.001

Tab. A.2: *The minimum adequate model of birth time effects on the hatching time of eggs laid early and late in a female's lifetime. The analysis is from a survival model using a Weibull distribution. Batch corresponds to an early or late birth time in the mother's life-cycle.*

	Df	Deviance	Resid. Deviance	$Pr(\chi^2)$
NULL			42455.16	
egg batch	1	8.65	42446.51	0.003274
juvenile food	3	28891.4	13555.12	0
log(juvenile density)	1	7977.92	5577.2	0
log(age-at-maturity)	1	1312.43	4264.78	0
juvenile food:log(juvenile density)	3	591.31	3618.59	0
egg batch:log(juvenile density)	1	16.82	3618.59	4.12E-05

Tab. A.3: *The minimum adequate model for the percent recruitment of adult mites from juvenile stage. Percent recruitment was analysed with a generalised linear model with binomial errors. The residual deviance for the last two terms are the same because the table was computed using sequential sums of squares and the deviance and significance of each of these higher order terms was estimated independently.*

	Df	AIC	LRT	$Pr(\chi^2)$
NULL		4810.289		
strata(rearing conditions)	1	4842.787	34.49762	0
adult density:delay:food	2	4813.585	7.29546	0.02605
rearing conditions:delay:adult food	1	4813.509	5.21975	0.022332
rearing conditions:adult density:adult food	2	4815.79	9.50097	0.008648

Tab. A.4: *The minimum adequate model for density, food amount and food delay effects on adult female survival. The model used survival analysis of the time of recruitment for individuals in specific treatments. The model is based on a normal distribution. The table reports the likelihood ratio tests (LRT) for the higher order terms evaluated as the last term in the model using sequential sums of squares.*

B. PAPERS

I was involved in the planning and conduct of the experiment with the other authors of the paper and the technical report. However, for my thesis, all analysis of the pooled data was conducted by me and independently from the analysis conducted and reported elsewhere.

Talkin' 'bout My Generation: Environmental Variability and Cohort Effects

Andrew P. Beckerman,* Tim G. Benton,† Craig T. Lapsley,‡ and Nils Koesters§

Institute of Biological Sciences, University of Stirling, Stirling FK9 4LA, United Kingdom

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ABSTRACT: In variable environments, it is probable that environmental conditions in the past can influence demographic performance now. Cohort effects occur when these delayed life-history effects are synchronized among groups of individuals in a population. Here we show how plasticity in density-dependent demographic traits throughout the life cycle can lead to cohort effects and that there can be substantial population dynamic consequences of these effects. We show experimentally that density and food conditions early in development can influence subsequent juvenile life-history traits. We also show that conditions early in development can interact with conditions at maturity to shape future adult performance. In fact, conditions such as food availability and density at maturity, like conditions early in development, can generate cohort effects in mature stages. Based on these data, and on current theory about the effects of plasticity generated by historical environments, we make predictions about the consequences of such changes on density-dependent demography and on mite population dynamics. We use a stochastic cohort effects model to generate a range of population dynamics. In accordance with the theory, we find the predicted changes in the strength of density dependence and associated changes in population dynamics and population variability.

Keywords: cohort effects, delayed density dependence, population dynamics.

Variation is a persistent feature in the environment of organisms. The environment experienced by one stage or age of an organism will therefore differ from that experienced in subsequent stages or ages. In such a variable environment, the conditions at one time can influence life-history traits and performance of individuals at future times and, subsequently, population dynamics (Beckerman et al. 2002). Within a generation, such delayed life-history effects can lead to demographic patterns such as cohort effects. Maternal or paternal environmental effects occur when delayed life-history effects cross generations.

Delayed life-history effects occur within generations across a wide range of taxa (Roff 1992; Lindstrom 1999; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002). Environmental conditions experienced early in life can lead to performance differences among well-defined cohorts within a population. Cohort effects arise when the variance in a life-history trait within a group of individuals in a population is significantly smaller than the variance in the trait among the population, thus making cohorts statistically distinguishable from each other. They are population-level responses to common environmental conditions and are a classical expression of delayed life-history effects in age- or stage-structured populations.

Examples of cohort effects come from life-history based studies (Albon et al. 1987; Oghushi 1991; Post et al. 1997; Lindstrom 1999; Metcalfe and Monaghan 2001; Lummaa and Clutton-Brock 2002; Wacker and von Elert 2002) as well as studies linking life history, age structure, and population dynamics (Stenseth et al. 1999; Coulson et al. 2001). More recently, there has been an interest in how cohort effects can generate and interact with variability in population dynamics (Beckerman et al. 2002; Lindstrom and Kokko 2002). In all of these discussions, delayed life-history effects are synchronized across cohorts and by definition influence life history and population dynamics by modifying the variability of density dependence among cohorts in a population.

Population Dynamic Theory of Cohort Effects

Lindstrom and Kokko (2002; their fig. 4) showed that when density dependence acts in a cohort-specific manner,

* Corresponding author; e-mail: apb1@stir.ac.uk.

† E-mail: tgb1@stir.ac.uk.

‡ E-mail: ctl1@stir.ac.uk.

§ E-mail: nk1@stir.ac.uk.

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a cohort effect can introduce variation among cohorts into the population. Their model provides the clearest explanation of how variation in density dependence at the population level can be generated by cohort effects. As a simple example, they imagined that 50% of the individuals in a population (cohort 1) could be of better quality and 50% (cohort 2) could be of worse quality than average. If we assume a nonlinear relationship between performance and density, then variation among cohorts along this curve can lead to performance levels that deviated from what might be expected in the population. This deviance is due to the geometry of density dependence (Lindstrom and Kokko 2002), where the vital rate in the population, averaged over cohorts, is either greater or less than the expected midpoint on the density-dependent function. This can happen because a chord between points on a density-dependent curve that represent different cohorts cuts the corner of the curve.

This averaging across cohorts can produce a different pattern of density dependence in the population from the one that assumes all individuals are equal. The direction of the deviation due to cohort effects is specified by the underlying shape of density dependence. If the underlying density dependence is concave (e.g., a saturating function), the average rate among cohorts can be less than would be expected from the underlying shape (a chord between points on the curve for each cohort falls below the curve at the given total density). If the underlying density dependence takes a convex shape (e.g., an exponentially decaying function), then a cohort mixing average can be higher than the underlying rate. The primary consequence of these effects is a more linearized relationship between the vital rate and density at the population level (Lindstrom and Kokko 2002).

From this demographic perspective, delayed life-history effects and patterns such as cohort effects can be a key process influencing variability, stability, and the way that delayed density dependence influences population dynamics (Beckerman et al. 2002). Lindstrom and Kokko (2002) showed that when underlying population dynamics are stable, cohort effects can increase population fluctuations in much the same way that introducing environmental variation would. However, cohort effects can decrease temporal variability in population dynamics when the underlying dynamics are variable, as they introduce a shallower form of density dependence than that generating the fluctuations in the first place.

In this study, we focused on the role that environmental variation, at various life stages, can have on shaping density dependence among cohorts in a model organism, the soil mite *Sancassania berlesei*. As there is now a theory about the demographic and population dynamic consequences of cohort effects, we experimentally examined patterns of density dependence among cohorts of mites exposed to various environmental conditions. Then, using this system-specific demographic data, we developed a cohort effects population dynamics model of the mites to assess the hypotheses about variability and stability implicit in the Lindstrom and Kokko (2002) theory.

Our experiments used individuals from laboratory stock populations of *S. berlesei* and employed a factorial experimental design that systematically varied the offspring and parental environment (see fig. 8 in the online edition of the *American Naturalist*). The range of treatments varied density and food availability at various points in the life cycle, corresponding to changes that populations can experience in a variable environment (e.g., Benton et al. 2001, 2002*a*). First, we examined the effects of environmental conditions on juvenile performance during early development. We examined the effects of initial density and patterns of food availability on the age at maturity, the proportion recruiting, size at maturity, and the trade-off between size at maturity and age at maturity.

Second, we examined the effects of environmental conditions during juvenile development on the expression of adult vital rates when the adult environment also varies. In particular, we examined the interaction between juvenile rearing conditions and adult density and food availability on adult survival, fecundity, and the egg size–egg number trade-off. These two sections comprise a comprehensive, longitudinal assessment of delayed life-history effects and can identify plasticity in life-history traits that arises from current and historical environments. Finally, having demonstrated the potential for cohort effects and developed hypotheses about the consequences of among-cohort averaging on population dynamics (Lindstrom and Kokko 2002) in the soil mites, we developed an age-structured model of mite dynamics to demonstrate the consequences that such cohort effects can have in this model system.

Methods 1: Early Juvenile Conditions and Juvenile Performance

The data reported in this section emerge from observations of juveniles during a longitudinal experiment that covers almost two generations (see app. A for full design and analysis details and fig. 8). To begin the longitudinal experiment, eggs from second-generation mothers reared from stock cultures were assigned to two parental rearing treatments (good or bad). On maturation to adulthood, males and females were paired randomly in three parental densities (1, 20, 50 pairs) and then assigned randomly to two parental food amounts (low and high) and two parental food timings (no delay or a 5-d delay in receiving food after maturation). Eggs from these parents were then

collected and subjected to a range of offspring treatments. Newly hatched offspring were subjected to the range of juvenile densities that they were born into and subsequently assigned randomly to high and low juvenile food amounts that were delivered with two juvenile food timings throughout their development (over time or in one pulse at hatching).

This factorial design allows a description of how cohort level variation in vital rates can arise in a population. We treat groups of mites as cohorts and examine their vital rates under different environmental conditions that are defined by current and historical treatments. From this we show life-history plasticity can lead to cohort effects and how the averaging effect specified by Lindstrom and Kokko (2002) can manifest itself in the soil mites. Now we document how conditions during early development affect juvenile performance.

Results: Juvenile Age at Maturity

We analyzed age at maturity using survival analysis on data specifying the time of death of individuals experiencing different combinations of food and density. Density was a continuous covariate (maximum density), and food was a four-level factor determined by the combinations of the two food manipulations above (high pulse, high over time, low pulse, low over time; see app. A).

The age-at-maturity data were fitted best by a Weibull distribution, and the interaction between density and food was significant ($\chi^2 = 3{,}033$, df $= 3$, $P < .001$). The effect of increasing density was to extend the age at maturity (fig. 1, panel $A = 50$ juveniles through to panel $D = 2{,}000$ juveniles). The effect of pulsed food, and in particular low amounts of pulsed food, was also to extend the age at maturity. The extension of age at maturity by pulsed food was substantially stronger as juvenile density increased, leading to the interaction.

According to the cohort theory presented above (Lindstrom and Kokko 2002), cohort variability could linearize density dependence at the population level. To see this pattern in the density dependence, we have plotted the median half-lives from the different food availabilities against density (fig. 1*E*). This characterizes both the underlying shape of density dependence and the range of median age at maturities (half-lives) that could exist among cohorts and lead to the effect suggested by Lindstrom and Kokko (2002). As the density dependence in age at maturity is concave, the effect of variation among cohorts in a population can be to lower the age at maturity over moderate densities.

Results: Percent Recruitment

Recruitment rates were analyzed using a generalized linear model with binomial error structure. As with age at maturity, the interaction between density and the four-level factor describing food availability for recruitment was significant ($F = 9.33$, df $= 3$, $P < .001$; model controls for age at maturity: $F = 21.30$, df $= 1$, $P < .001$; *F*-test assumes quasi-binomial family and is suitable for overdispersed binomial data). Food delivered over time always resulted in at least 40%–50% recruitment, while a pulse of food at hatching severely reduced recruitment to near zero at high densities. High amounts of food delivered in a pulse resulted in greater recruitment, especially at low densities, where recruitment rates approached those for food delivered over time. Again, there is a convex pattern of density dependence in percent recruitment for each of the four food treatments (fig. 1*F*). As the relationship between density and recruitment is always convex, the predicted averaging effect of variation among cohorts in a population is to increase recruitment over moderate juvenile densities.

Results: Juvenile Size at Maturity

Food and density also interact to affect size at maturity (generalized linear model with Gaussian errors; $F_{\text{food} \times \text{density}} = 10.26$, df $= 2, 23$, $P < .001$). Increased density reduced size at maturity and more so when food was limited (fig. 2). Pulsed food seemed to reduce density dependence by virtue of forcing a possible minimum size at maturity across all densities (fig. 2; see app. A for justification of pooled pulsed food treatments). Again, the relationship between size and density is convex, suggesting that variation among cohorts in a population could lead to increased size at maturity over moderate densities and a more linear relationship in the density dependence.

Results: Age- and Size-at-Maturity Trade-off

In order to examine the age- and size-at-maturity tradeoff, we linked data from two separate experiments. First, we gathered data on age at maturity and size at maturity from the longitudinal, factorial experiment (app. A). These data contain information on the slope of the trade-off under restricted food and a wide range of densities. This was combined with data from a separate experiment that varied only food levels, holding density constant. Per capita food was much higher in the separate experiment, and density was fixed (see app. B for experimental design).

We used the data from both of these experiments to describe the changes in the slope of the relationship between the mean size and age at nine different food levels—

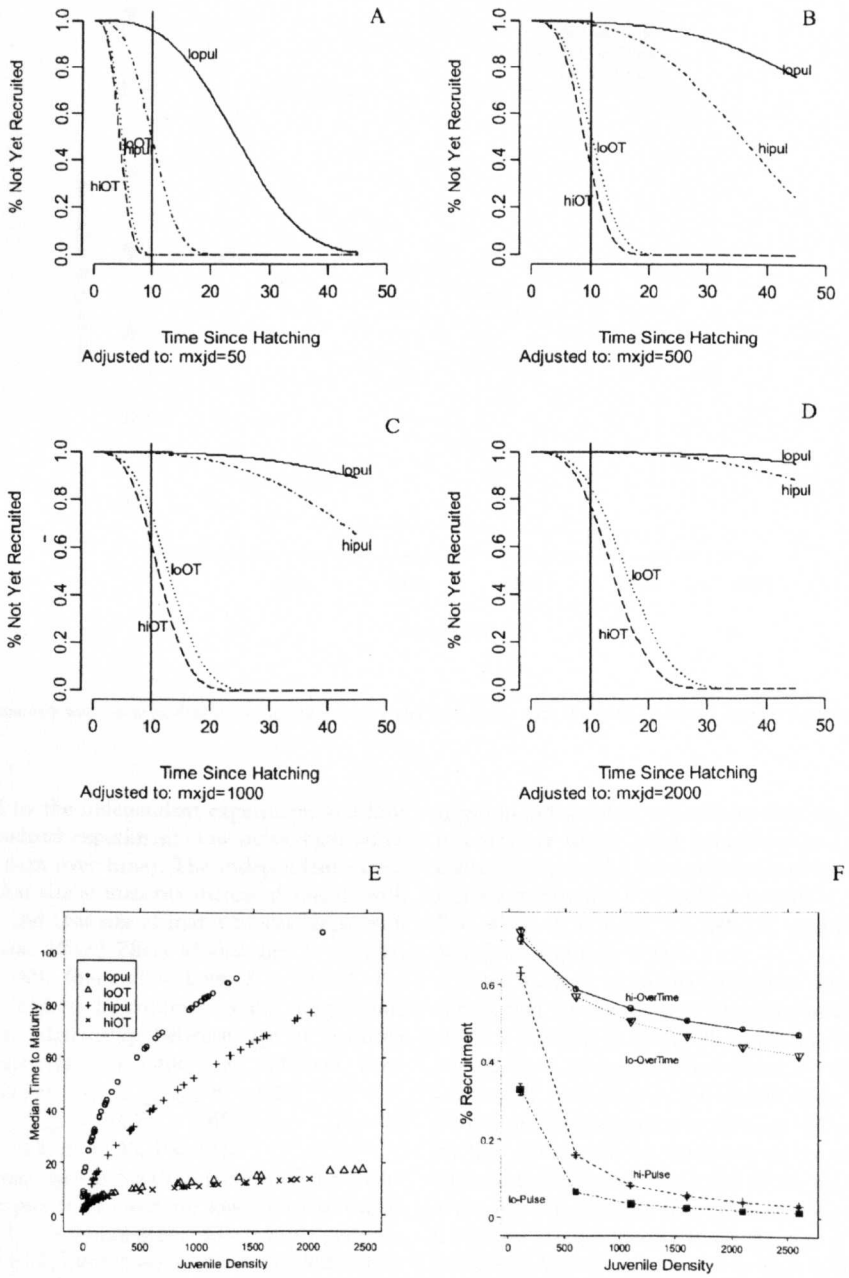


Figure 1: Density dependence in age at maturity and percent recruitment. *A–D*, Density dependence in age at maturity can be shown by plotting recruitment curves versus time for a variety of densities. Moving from density 50 to 500 to 1,000 to 2,000, *A–D* show that age at maturity arises from an interaction between density and food delivery during juvenile development. The vertical line at age = 10 in each panel makes clear the increases in age at maturity due to density and to food. *E*, The cohort-averaging hypothesis is dependent on the shape of density dependence among cohorts. Each curve represents variation in median age at maturity (age) for the different food regimes (see text and app. A). Consistently convex shapes predict that variation among cohorts in a population could lead to an earlier age at maturity. *F*, Percent recruitment was a concave function of juvenile density and food levels. The panel shows the effect of the juvenile food × juvenile density interaction, having controlled for the effect of age at maturity on recruitment. Each line corresponds to a particular feeding regime. Pulsed food (*pul*) was delivered only at maturation, while over-time food (*ot*) was delivered continuously over the development period (app. A); *lo* and *hi* correspond to low and high food levels.

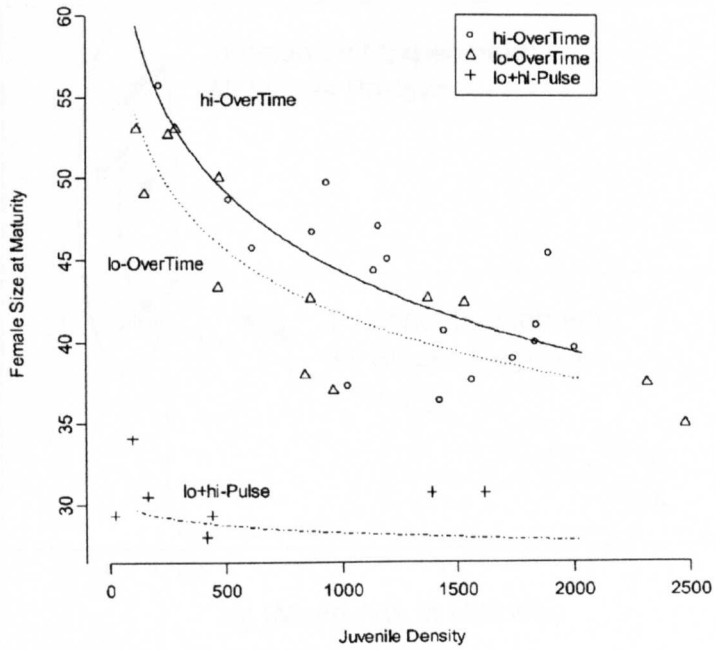


Figure 2: Size at maturity was a convex function of juvenile density and food levels. Each line is the predicted density-dependent relationship for a given food level.

five determined by the independent experiment and four from the longitudinal experiment (low pulse, high pulse, low over time, high over time). The independent experiment showed that size at maturity increased linearly with age at maturity and that size at maturity was larger with more food (Linear Mixed Effect Model: age, $F = 12.41$, df $= 1, 98$, $P < .001$; food, df $= 1, 98$, $F = 206.58$, $P < .0001$; app. B). This is in contrast to the longitudinal data, where the relationship between age at maturity and size at maturity was either not different from zero or negative ($r_{\text{low+high pulse}} = -0.20$, df $= 7$, $P = .6$; $r_{\text{low overtime}} = -0.75$, df $= 11$, $P < .01$; $r_{\text{high overtime}} = -0.68$, df $= 15$, $P < .01$).

These combined data generate a picture of the trade-off: when per capita food levels are low (factorial experiment data; fig. 3), we would expect food supply patterns, along with density, to govern age at maturity. Under these conditions, size should increase marginally with age from an asymptotic minimum as food levels gradually increase (density drops; shallow negative slopes from factorial data). As food increases, the age at maturity is reduced faster than size increases until a minimum age is met. At this point, with more ample food supplies, mites can increase their size at a faster rate relative to age (steep positive slopes in independent experiment data; fig. 3). Thus, there is a strategy under good conditions to trade off age at maturity to gain size. As fecundity is proportional to body volume (fecundity $= -33.25 + 0.0044 \times$ female volume; $F = 48.49$, df $= 1, 55$, $P < .001$, $R^2 = 0.47$), this can be beneficial if growth is fast.

If we assume, as in the univariate density-dependent curves, that the convexity or concavity of the trade-off can define a location in trade-off space, this shape can cause one of three outcomes when there is variability among cohorts. If cohorts are distributed along the lower flat portion of the trade-off, the average will lie more or less on this portion of the trade-off. If the cohorts are distributed along the lower portion of the inflection area, the averaging will increase size more than age. Alternatively, if cohorts are distributed along the upper portion of the inflection, age can increase more than size.

Our data suggest that the initial density and food availability that juveniles experience can structure where cohorts realize age and size along the trade-off—there is plasticity in vital rates, dependent on conditions at hatching, that alters the trade-offs that can govern the life history later in life. Moreover, the effect of multiple cohorts in a population can have a variety of effects on the population,

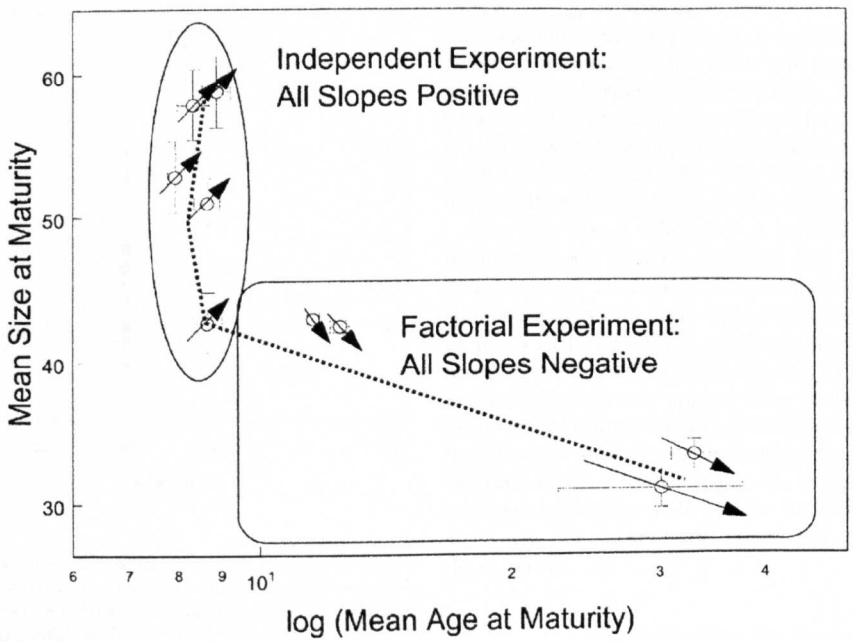


Figure 3: By combining data from two experiments on the relationship between size at maturity and age at maturity, it is possible to generate the shape of the trade-off between the two traits. Arrows correspond to the direction of change in each data set, leading to a C-shaped trade-off function (*dashed line*, for reference only) where high food levels (independent experiment) allow for increases in size and age, while poor food conditions predict decreasing size with increasing age.

the effect depending on where environmental variability introduces variation among cohorts.

Methods 2: Early Juvenile Conditions and Adult Performance

The data reported in this section are from the first half of the longitudinal data collected according to the design in appendix A (and see above). Above, we focused on how conditions during juvenile development can generate cohort effects in juveniles. Here, we focus on how these conditions interact with adult conditions to generate, extend, or ameliorate the cohort effects at the adult stage.

Adult survival, fecundity, and the egg size–egg number trade-off were measured in conditions dictated by parental rearing conditions prior to adulthood (good or bad), adult density (1, 20, 50 pairs), adult food amounts (low or high) and adult food timing (no delay or 5-d delay). As above, we treat groups of mites within a treatment combination as a cohort and examine the plasticity in vital rates under different treatment-specified environmental conditions. With these patterns, we develop a picture of potential cohort variation in adults and an understanding of how the averaging effect specified by Lindstrom and Kokko (2002) could manifest itself across adjacent life stages.

Results: Adult Survival

We analyzed adult (female) survival using survival analysis, having followed the fate of all females in a population over the 35-d period (max) that eggs were laid. With five potential factors in the model, we set three-way interactions as our maximum model order (app. A). We defined our minimum adequate model based on Akaike Information Criterion (AIC) values and then P values (app. A) and found that rearing × adult density × adult food ($\chi^2 = 27.01$, df = 2, $P < .001$) and adult density × adult food timing × adult food ($\chi^2 = 7.26$, df = 2, $P = .026$) were significant interactions. Adult rearing thus has a significant effect on adult female survival by interacting with current adult conditions. This is clearly a delayed life-history effect. The survival curves (see fig. 9 in the online edition of the *American Naturalist*) show that the effect of adult density is not consistent and depends on rearing conditions—those set during early development. Under good conditions—good rearing, no delay, and a lot of food—higher

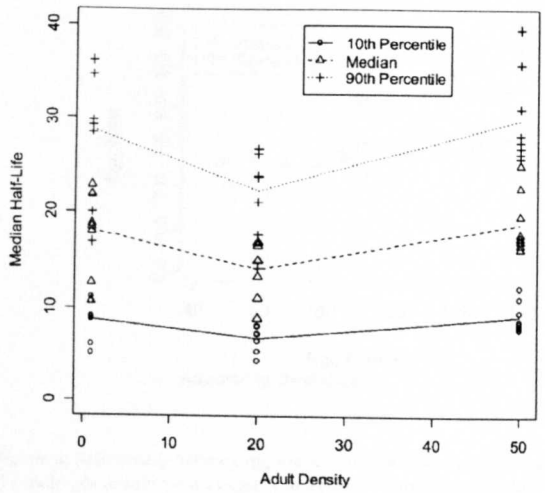


Figure 4: Shape of density dependence in survival of adults is a shallow dip. Each line and set of points present the 10th, 50th, and 90th percentiles of the survival curves for females versus adult density. The convex nature of the relationship predicts an increase in time to death when there is variability among cohorts in a population.

densities extend life as competition and reproduction interact (fig. 9*A*). Low densities result in longer lives, though, when food is low (fig. 9*B*, 9*D*, 9*F*), and this again is mediated by adult rearing conditions and current adult food levels.

The quantiles (e.g., median time to death) of survival plotted against density (fig. 4) show that the relationship between survival and density is shallow but concave, with longer lives commonly occurring at low and high densities and a minimum at moderate densities. Based on the theory presented in Lindstrom and Kokko (2002), this would suggest that in a population with multiple cohorts, the averaging effect would tend to extend life for the females. However, the interaction plots (fig. 9) show that a wide range of rearing, density, and food conditions can result in very similar death times. Cohort variation in a population among these similar rates that arises for different reasons could lead to very little change in the average life span.

Results: Fecundity

Fecundity was measured as the averaged daily per capita fecundity (eggs/female/tube/d). Based on our minimum adequate model, we found that rearing, adult density, and the delay in feeding were the most influential variables for fecundity ($F_{\text{delay}} = 33.31$, df $= 1, 40$, $P < .001$; $F_{\text{rear} \times \text{density}} = 5.36$, df $= 2, 40$, $P = .008$). Good rearing conditions tend to increase fecundity, but high adult density can ameliorate this effect and make fecundity independent of rearing conditions (see fig. 10*A* in the online edition of the *American Naturalist*). Thus, conditions during development only appear to affect fecundity when the population size of adults is low. There is a delayed life-history effect that is conditional on current conditions.

Note too that a delay of 5 d in feeding adults after maturity always reduced fecundity (fig. 10*B*), indicating that cohorts of adults that face adverse conditions at the start of maturity may perform very differently than cohorts of adults that mature into good conditions. This is a delayed life-history effect that originates in the adult stage. Fecundity and density appear to be related in a negative exponential fashion (convex), indicating that fecundity can increase over moderate densities when there is intercohort variation in the population (fig. 5), though, like the size- and age-at-maturity trade-off, the increase could be very small on the near linear right tail of the density dependence.

Results: Egg Size–Egg Number

The egg size–egg number trade-off was examined in an experiment separate from the factorial, longitudinal experiment. To examine the trade-off, we crossed two juvenile rearing conditions (good and bad) with two adult current food conditions (high and low) and measured the relationship between egg size and number. We used

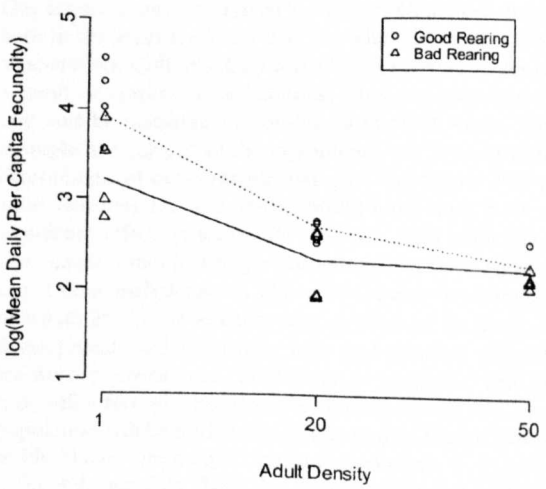


Figure 5: Density dependence in per capita fecundity is convex, predicting that if cohort variation exists, density dependence would be linearized when fecundity increases over moderate densities.

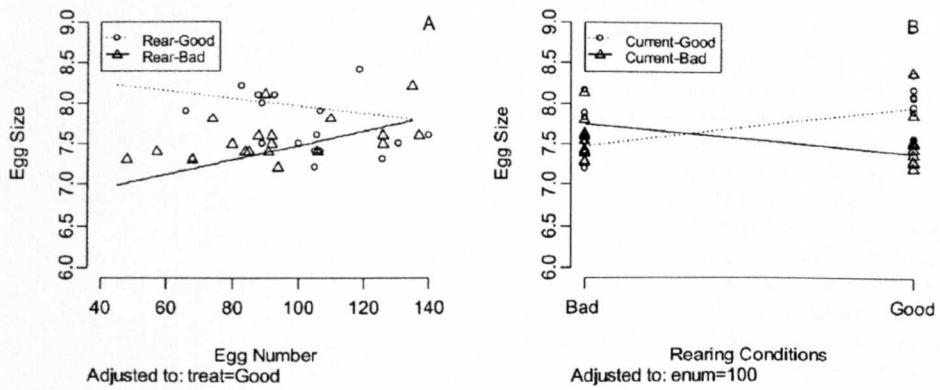


Figure 6: Relationship between egg size and number was related to rearing conditions and to current conditions. Bad rearing conditions (*A*) caused the trade-offs to shift from a negative to a positive slope. An interaction between rearing and current conditions (*B*) affects egg size and demonstrates a possible invariant egg size in the population when cohort variability exists in a population.

ANCOVA (see app. B for experimental details) to investigate the patterns. We found that good rearing conditions generated a negative relationship between size and number of eggs, while poor rearing resulted in a positive egg size–egg number relationship ($F_{\text{rearing conditions} \times \text{egg number}} = 11.53$, df = 1, 31, $P = .0019$; fig. 6*A*). Current conditions did not change the slope of the trade-off; a combination of good current food and good rearing food leads to higher egg numbers than any combination experiencing a bad condition ($F_{\text{rearing conditions} \times \text{current conditions}} = 18.65$, df = 1, 31, $P < .001$; fig. 6*B*). The contributions of rearing and current treatments were assessed by examining this model when either the rearing or the current treatment was removed. When rearing conditions were removed, the adjusted R^2 was reduced from 45% to 4%, while when current conditions were removed, the adjusted R^2 was reduced from 45% to 14%. This shows that both current and rearing conditions play a major role in explaining variation in this trade-off.

Thus, our data show that environmental conditions during early development can influence a key trade-off in adult life histories. Rearing conditions and current conditions combine to influence reproductive allocation decisions. By determining numbers (offspring density) and size (offspring quality), the egg size–egg number trade-off is a route by which cohort effects could be transmitted across generations as a maternal effect. Moreover, as with the size- and age-at-maturity trade-off, we can hypothesize what intercohort variation along this trade-off might generate at a population level. The answer lies in examining the rearing food × current food plot (fig. 6*B*). Given the nature of the interaction, it appears that any intercohort variation in egg size and egg number would lead to an invariant relationship in the size of eggs at the population level because the averaging of sizes among these conditions could have a slope of zero.

Experimental Summary: Delays, Cohort Effects, and Population-Level Hypotheses

Our experimental data lead to four conclusions. First, early developmental conditions for juveniles can affect juvenile performance and adult performance: the plasticity in demographic traits is sensitive to historical environments. Our results show that delayed life-history effects can occur both in the short term and over the whole lifetime within a generation. Cohort effects can arise in juvenile stages as a result of conditions at hatching. They can also extend into and be mediated by conditions in adult stages. For example, the current adult environment can be a stronger determinant of performance than past conditions. When adult densities are low, poor rearing conditions have a significant effect on fecundity. However, high adult densities cause a much stronger reduction than poor rearing can at these high densities. Thus, the sequence and timing of variability in the environment, relative to the path of development, will determine how and whether delayed life-history effects arise (Beckerman et al. 2002). This in turn will affect whether patterns of cohort variation in a population will be statistically visible against the backdrop of life-history plasticity to current conditions.

Second, our data show that cohort effects are not restricted to early developmental conditions. A delay in obtaining food at the initiation of adulthood had a severe impact on measures of fecundity and adult survival, highlighting that cohort effects can arise anywhere in the life

cycle. While the emphasis to date has been on early development (Lindstrom 1999; Metcalfe and Monaghan 2001; Beckerman et al. 2002; Lummaa and Clutton-Brock 2002), certain stage- or age-structured life histories could contain mature stages and ages that are sensitive to environmental conditions (e.g., maturation or metamorphosis). These adulthood effects can also be perpetuated across the rest of the life span and potentially across subsequent generations (A. P. Beckerman, T. G. Benton, C. T. Lapsley, and N. Koesters, unpublished manuscript).

Third, these data demonstrate that trade-offs are also sensitive to conditions during development. This is not surprising given that trade-offs are composites of univariate fitness measures that can be independently sensitive to environmental conditions. However, when a life history is viewed through its trade-offs, the types of predictions that can be made about the consequences of intercohort variation may be different from the types of predictions made from the univariate measures (see below).

Finally, our data demonstrate a wide range of predictions about the consequence of intercohort variation on density-dependent rates and evolutionarily significant trade-offs. Lindstrom and Kokko (2002) hypothesized that intercohort variation in a population could linearize nonlinear density dependence, and our data show that there is enough plasticity in mite vital rates for this to happen in mite populations. Density dependence in our data takes three forms: saturating and increasing (always concave; e.g., age at maturity, fig. 1*E*), asymptotic decreasing (always convex; e.g., % recruitment, fig. 1*F*; fecundity, fig. 5), and hyperbolic with a local minimum (extreme convex; e.g., adult survival, fig. 3). According to the theory, convex surfaces tend to increase vital rates at moderate densities while concave surfaces decrease them. Thus, in a mite population experiencing a variable environment, intercohort variation could lead, on average and over moderate densities, to earlier age at maturity, higher recruitment, larger size at maturity, a longer life span for adults with increased per capita fecundity, and lifetime reproductive success. These increases or decreases would tend to occur over moderate density ranges and correspond to a linearization of density dependence across these vital rates.

It is important to realize that fitness and population dynamics are unlikely to be defined by single measures of plasticity in individual traits. Fitness and population dynamics are an integrated outcome of many traits, the former in terms of trade-offs and the latter in terms of density dependence. Applying the "averaging" logic to the age- and size-at-maturity trade-off, we can see that the trade-off shows a far richer range of outcomes from environmental variation than its component univariate measures due to the complexity of its shape. Variation among cohorts that occurs within the range of relatively poor conditions (right asymptote, fig. 3) can have little or no effect on the size-age relationship, while variation around slightly better conditions (inflection area, fig. 3) can increase size but not age. Under good conditions, size could decrease with age increasing. Alternatively, drastic variation in the environment leading to cohorts at either end of the trade-off could lead a linearized and simple negative relationship between size and age. The range of predictions that can be made about the population-level consequences of intercohort variation requires an understanding of the timing and range of environmental conditions that could be (and are) experienced by a population.

The invariant population level–egg size relationship that can result from intercohort variability in the mites could have substantial fitness consequences as well. As egg size is positively related to subsequent adult size ($F = 139.3$, $df = 2, 62$, $P < .001$, $R^2 = 0.82$) and adult size is positively related to subsequent fecundity ($F = 48.49$, $df = 1, 55$, $P < .001$, $R^2 = 0.47$), intercohort variability that can lead to invariant egg sizes can generate a near constant average population reproductive output (population level), though with large amounts of variability. However, this depends on temporal patterns of environmental variation. A sequence of temporally autocorrelated environmental events will not lead to invariant measures of egg size unless generation times, and thus the persistence of cohorts in a population, are longer than the window of variability.

An Age-Structured Model with Cohort Effects

Using the results above, we developed a five-stage model of mite dynamics that corresponds to the five life stages of the mites. Our objective was to generate replicate, hypothetical time series from scenarios incorporating the effects of birth year density and birth year quality, two measures of environmental quality that can influence the formation of cohort effects. We then used these time series to estimate the effects of intercohort variation on the density-dependent exponent of fecundity and recruitment. These changes, and their associated population dynamics, were compared to expectations from previous theory (Lindstrom and Kokko 2002) that predict changes in the exponents (see "Population Dynamic Theory of Cohort Effects").

Our time series were generated following the process implemented by Lindstrom and Kokko (2002), but our model had more structure and compared between the effects of birth year density (traditional delayed density dependence linked to birth year) and birth year quality (density is current, but the exponent of density dependence is altered by birth year quality) under stochastic conditions. According to the theory presented by Lindstrom and Kokko (2002), it should be possible to statistically compare

the underlying deterministic exponent of density dependence to the values obtained statistically from replicate stochastic simulations and judge whether intercohort variation in a structured population can lead to estimates of density dependence that differ from underlying deterministic rates. Table 1 presents the density-dependent functions for fecundity, juvenile survival, recruitment, and adult survival, estimated from the density-dependent data collected in the experiments above. Our model had the following deterministic form:

$$E_{t+1} = \lambda^*_{t-i} A_t - \varphi^* E_{t-4}, \quad (1a)$$

$$J1_{t+1} = \varphi^* E_{t-4} - J1_t, \quad (1b)$$

$$J2_{t+1} = J1_t + \sigma_2^* J2_t - \rho^*_{2t-i} J2_t, \quad (1c)$$

$$J3_{t+1} = \rho^*_{2t-i} J2_t + \sigma_3^* J3_t - \rho^*_{3t-i} J3_t, \quad (1d)$$

$$A_{t+1} = \rho^*_{3t-i} J3_t + \sigma_a^* A_t, \quad (1e)$$

where λ was adult (A) per capita fecundity, φ was egg (E) hatch rate, σ_{1-3} were survival rates of juvenile (J) age classes 1–3, σ_a was adult survival, ρ_{1-3} were the recruitment rates of juvenile ages 1–3, and i was the estimate of hatch year for juveniles or maturation year for adults. We allowed lags to occur in both juvenile performance affected by conditions at hatching and in adult performance affected by conditions at maturity. The hatch year or maturity year (i) was estimated as the median time to leave an age class: 1/(recruitment rate + mortality rate) time steps previously.

For each of these lags, there were two routes for environmental quality to have an effect: via the quality of the hatch or maturation year and via the density in the hatch or maturation year. We implemented quality variation following the recipe of Lindstrom and Kokko (2002). We adjusted the exponent of density dependence in fecundity (λ) and recruitment (ρ) by a small positive or negative amount determined by the quality of that year $Q_t \sim N(0, \text{Var}_Q)$. Year quality generated density independent adjustments of the vital rates (+value = good year = weaker density dependence). The hatch year or maturation year density variation is classical delayed density dependence linked specifically to the density at hatch or maturity year. Fecundity (λ_{t-i}) and recruitment rates (ρ_{t-i}) are adjusted to hatch or maturation year density (i) before multiplying by current year density.

These two routes represent distinct measures of quality with independent mode of action on performance. Density-independent factors can improve or reduce performance and are modeled by year quality. Alternatively, historical variation in density can characterize good or bad years and lead to future density-dependent responses; these are modeled by classical delayed density dependence. For all models, we also include environmental stochasticity ($\varepsilon_t \sim N(0, \text{Var}_\varepsilon)$) that adjusts population sizes in the current year and was added linearly on a log scale (table 1; Dennis et al. 1995). Note that juvenile class one matures as a cohort each year and that egg hatching is on a 4-d delay as the median time to hatch is 4 d.

The deterministic dynamics of this model are decaying oscillations; this is supported in long-term experimental times series (Benton et al. 2001). Using this model, we generated 100 time series of 1,000 time steps from each of four stochastic scenarios: (1) control: stochastic density dependent (DD), where the model (eqq. 1) is run with no delays ($i = 0$); (2) delayed quality dependence (DQ), where the exponent of current year density dependence is adjusted by hatch or maturity year quality; (3) delayed density dependence (DDD), where the current year density dependence is adjusted by the exponent of current year density dependence is adjusted by hatch or maturity year density; and (4) delayed density and delayed quality dependence (DDDQ), a combination of delayed effects linked to hatch/maturity year quality and hatch/maturity year density.

The deterministic rate equations (table 1) provide baseline estimates of the exponents for fecundity and recruitment. We used nonlinear regression to estimate the intercept of the exponential density-dependent functions from each of the 100 stochastic simulations from each scenario. We then used t-tests to make comparisons among scenarios. Based on the Lindstrom and Kokko theory

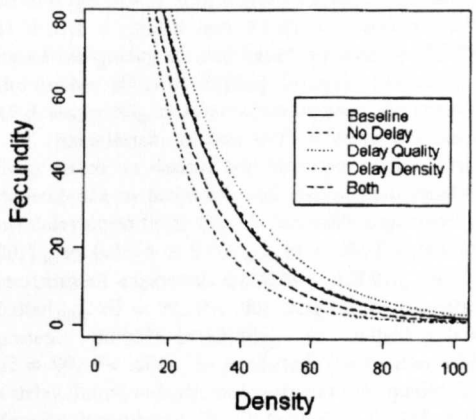
Table 1: Vital rate equations used in the model

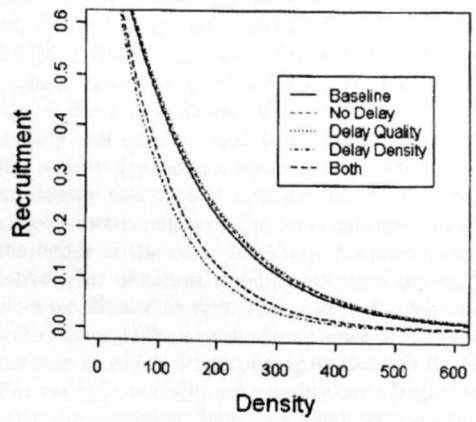
Symbol	Definition	Equation
λ	Fecundity	$1 + 200 \times \exp(-.05 \times \text{total density})^a$
φ	Hatch rate	.2
σ_j	Juvenile survival	$.93 + .01 \times \text{juvenile density}/(1 + .011 \times \text{juvenile density}^2)^b$
ρ	Recruitment	$.9 \times \exp(-.007 \times \text{juvenile density})$
σ_a	Adult survival	.85

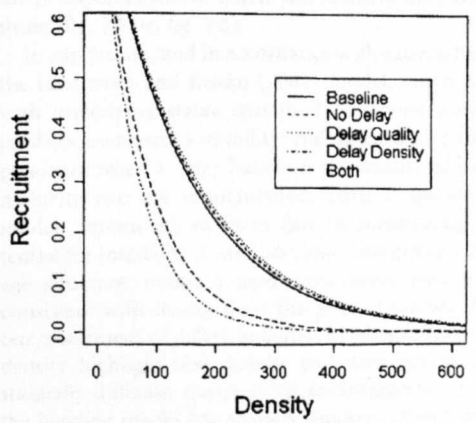
Note: Rate equations are fitted functions to density-dependent data from the cohort experiments.

[a] Total density = $A_t + 1/3 \times (J1_t + J2_t + J3_t)$.

[b] Juvenile density = $J1_t + J2_t + J3_t + 3 \times A_t$.







(Lindstrom and Kokko 2002) and the convex shape of our fecundity and recruitment functions, we made three predictions. First, a delay based on year quality (DQ) should increase the coefficient of variation in the population dynamics relative to the baseline (DD) model. Second, a delay based on year quality (DQ) should linearize the density dependence in fecundity and recruitment compared to the baseline model (DD). Finally, if a delay based on density (DDD) generates cycles and subsequently stronger estimates of density dependence, the further addition of a delay based on quality (DDDQ) should linearize density dependence more than it did without a delay based on density (DQ) because the chord connecting cohort values cuts a steeper line (see "Population Dynamic Theory of Cohort Effects").

Model Results

Cohort effects implemented as delayed quality dependence (DQ) and delayed density dependence (DDD) have significant effects on the estimates of the slope of fecundity and recruitment in our model (fig. 7; see also fig. 11 in the online edition of the *American Naturalist*). As predicted, a delay based on birth year quality linearized the density dependence in fecundity (fig. 7*A*; mean exponent ± 95% confidence interval [CI]; $0.049 \pm 0011_{DD}$ vs. $0.044 \pm 0.002_{DQ}$, df = 99, $P < .001$) and recruitment (fig. 9*B*, *C*; stage two: $0.00676 \pm 0.0001_{DD}$ vs. $0.00664 \pm 0.0002_{DQ}$, df = 99, $P < .001$; stage three: $0.00676 \pm 0.0001_{DD}$ vs. $0.00657 \pm 0.0001_{DQ}$, df = 99, $P < .001$). While these are significant but biologically small changes in the slope, they were capable of generating a significant increase in the coefficient of variation in the population ($0.2317 \pm 0.0003_{DD}$ vs. $0.268 \pm 0.002_{DQ}$, df = 99, $P < .001$).

A delay linked to hatch or maturity year density generated a significant shift in dynamics (fig. 11). Autocorrelation function patterns moved from a linear decay to 0 at 50 d (DD; consistent with decaying oscillations) to an exponential decay to 0 at 60 d (DQ) to a cyclic pattern with a 42-d cycle (DDD); generation times moved from

Figure 7: We generated data from four scenarios (stochastic density dependence, delayed quality dependence, delayed density dependence, delayed density and delayed quality dependence) in our simulation model. For each scenario, 100 times series of 1,000 time steps were used to estimate 100 density-dependent exponents of fecundity and recruitment. Each panel presents the baseline shape (*solid line*; see table 1) for the density dependence and then the shapes generated by the estimated shift in the exponent of density dependence corresponding to the four models: *A*, fecundity; *B*, stage two juvenile recruitment; *C*, stage three juvenile recruitment. Confidence intervals and significance tests are presented in the text.

an average of 8 ± 0.16 d (DD: mean ± CI; max 15 d) to 11 ± 1.16 d (DDD: max 23 d). The addition of a delay based on quality to one based on density (DDDQ) had the further effect of shifting the cyclic period from 42 to 45 d, suggesting an interaction between the types of delay.

A characteristic of this shift in dynamics caused by a delay based on density was stronger estimates of density dependence in fecundity and recruitment relative to the no delay conditions (fig. 7; fecundity exponent: $0.049 \pm 0011_{DD}$ vs. $0.0658 \pm 0.002_{DDD}$, df = 99, $P < .001$; stage two recruitment exponent: $0.00676 \pm 0.0001_{DD}$ vs. $0.0108 \pm 0.0001_{DDD}$, df = 99, $P < .001$; stage three recruitment exponent: $0.00676 \pm 0.0001_{DD}$ vs. $0.0154 \pm 0.0002_{DDD}$, df = 99, $P < .001$). As predicted, the further addition of a delay linked to birth and maturity year quality linearized density dependence (fig. 7; fecundity: $0.0658 \pm 0.002_{DDD}$ vs. $0.057 \pm 0.0003_{DDDQ}$, df = 99, $P < .001$; stage two recruit: $0.0108 \pm 0.0001_{DDD}$ vs. $0.0098 \pm 0.0001_{DDDQ}$, df = 99, $P < .001$; stage three recruit: $0.0154 \pm 0.0002_{DDD}$ vs. $0.0126 \pm 0.0003_{DDDQ}$, df = 99, $P < .001$) and also increased variability ($1.83 \pm 0.1_{DDD}$ vs. $1.97 \pm 0.08_{DDDQ}$). Again, there is evidence of an interaction between the density and quality based lags: quality effects linearized the density dependence more severely when a lag based on density had made it stronger (fig. 7). Graphically, the chord between points on the curve cuts more steeply when the bends in the curve are sharp. Moreover, the quantitative effect of cohort variability differed among the three rates we allowed to vary. This is in part a response to the initial shape of the density dependence. It may also reflect the way in which delays propagate through the life cycle. For example, maturity year quality has a larger linearizing effect on fecundity than does hatch year quality on recruitment (fig. 7*A* vs. fig. 7*B*, 7*C*), and stage two recruitment responds less to hatch year density than does stage three (fig. 7*B* vs. fig. 7*C*).

In conclusion, and in accordance with expectations from the Lindstrom and Kokko (2002) model, our mite model with underlying stable dynamics (decaying oscillations) predicts increased variability and linearized density dependence when a delay based on the quality of hatch and maturity year are implemented. Thus, a species-specific model, parameterized from data demonstrating the potential for intercohort variation and containing substantial age structure, makes a prediction about mite dynamics consistent with theory from the general model. However, our assessment of delays linked to hatch and maturity year density highlight that density and year quality are substantially different measures of environmental quality. As the baseline model has shallow density dependence in fecundity and recruitment to begin with, the effects of cohort variation based on hatch and maturity year quality are small on their own. However, simulations with a delay based on density generated stronger density dependence than shown in baseline models with no delay. Subsequently, variation based on quality had a much larger effect when paired with variation based on density because the delay based on density increased density dependence. The effect of year quality (the chord effect) is thus very dependent on the shape/strength of density dependence and, as discussed above, the structure of the life cycle.

Summary

We have presented data and models from a laboratory system of soil mites showing that cohort effects, a form of delayed life-history response to historical environmental conditions, can be common, can reflect plasticity in the life history, and can affect population dynamics. Our data show first that cohorts can vary in response to conditions at hatching (early development) and that these responses can be mediated by conditions later in life. Second, they show that conditions at maturity can also generate "adult" cohort effects, thus generalizing the current focus in the cohort literature on early development. Third, the data support the Lindstrom and Kokko (2002) hypothesis that intercohort variation can linearize density dependence in many vital rates. Fourth, cohort-level variability in single life-history traits can lead to different conclusions about the effects of cohorts on density dependence than when considering trade-offs. Trade-offs show a far richer range of fitness-related outcomes when cohort variability exists. Fifth, our model predicts that the patterns suggested by Lindstrom and Kokko (2002) are qualitatively robust to changes in model structure, that birth year environmental quality and birth year density are two measures of environmental quality for a cohort with vastly different population dynamic effects, and that when age structure is present, cohort-level variation can have quantitatively different effects on recruitment and fecundity. Finally, the data and models demonstrate that cohort variability in a population with associated population dynamics can affect estimates of density dependence in a population. It is thus important to consider sources of variability, stage or age structure, and density-dependent mechanisms when estimating density dependence in a population.

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APPENDIX A

Experimental Design and Analysis

The life cycle of *Sancassania berlesei* consists of five stages: eggs followed by a larval, protonymph, and tritonymph stage, and then adulthood. Experimental mite populations are maintained in glass tubes (20 mm × 50 mm) three-quarters filled with plaster of paris that is kept moist to maintain humidity and kept at a constant 24°C in unlit incubators with food supplied in the form of granulated yeast.

The experimental design is a straightforward factorial expansion of four binary treatments, one three-level treatment, one four-level treatment, and one covariate. The experiment began with eggs drawn from long-running stock cultures initiated in 1996. The eggs, and then juveniles, were allowed to develop in one of two parental rearing conditions: good conditions defined by low densities (~20 mites) and ad lib. food or bad conditions defined by high densities (~100 mites) and restricted food. When the mites reached maturity, adults were paired into three parental density treatments (1, 20, or 50 pair), each replicated eight times at the level of rearing. Four of each set of eight tubes were then assigned to one of two adult food treatments (1 or 5 balls) and then two each of these to one of two adult food delay treatments (fed at pairing or 5 d after pairing). Thus, there are two replicates of each of the treatment combinations. This portion of the experiment allowed investigation of the effect of early development conditions on adult performance ("Methods 2: Early Juvenile Conditions and Adult Performance").

Eggs laid by these parents were used in our offspring experiments ("Methods 1: Early Juvenile Conditions and Juvenile Performance"). Eggs were collected at days 4–6 and 9–11. These two timings are considered parental age and are examined in A. P. Beckerman, T. G. Benton, C. T. Lapsley, and N. Koesters, unpublished manuscript on maternal effects. Four of the six sets of eggs (days 4, 5, 9, 10), comprising two replicates each, were randomly assigned a juvenile density score (maximum juvenile numbers hatching: 20–2,500) and then to one of four juvenile food treatments. Food was either pulsed at hatching or delivered over time throughout development, and it was provided ad lib. or in a limited manner. This led to four juvenile food treatments: low pulse, high pulse, low over time, and high over time.

All data were collected by counting individuals or eggs in the tubes under a stereo dissecting microscope. All statistical analyses were implemented in R (Ihaka and Gentleman 1996) and use the HMISC and DESIGN libraries (Harrell 2000) and the MASS library (Venables and Ripley 1999). We generated minimum adequate statistical models using the following steps (Crawley 1993). All models were initially specified at the three-way interaction level. Akaike Information Criteria were used to reduce this model. The use of AIC is effective for generating predictive models but can often retain effects that have P values greater than .05. From this point on, we reduced the model by hand using P values of .05 as a cutoff, thus generating a parsimonious explanatory model.

Survival data (juvenile age at maturity, adult survival) were analyzed with parametric survival models (our data do not conform to Cox-proportional hazard assumptions). Selection of the distribution was based on an examination of censored model residuals and was compared to theoretical null distributions (Harrell 2000). Recruitment data were analyzed using a generalized linear model with binomial error structure. F-tests were used to assess significance. An F-test in a binomial model assumes a quasi-binomial family and is suitable for overdispersed binomial data. All other rates were analyzed with ordinary least squares.

For female size, we aggregated data from low- and high-pulse food treatments. The two treatments contain less than five data points each, and based on similarities among pulsed treatments in other analyses, we felt that we could increase our power and decrease the possibilities of influential points by lumping these two groups.

APPENDIX B

Trade-offs and the Egg-Adult-Egg Loop: Age at Maturity and Size at Maturity, Egg Size–Egg Number, Egg Size–Adult Size, and Adult Size Fecundity

In order to get a better view of the influence of past and present food conditions on the relationship between size at maturity and age at maturity, we assessed data from the longitudinal experiment and the following complementary experiment. We crossed two adult rearing conditions with five adult food amounts holding density and the timing of food constant. Twenty eggs were collected from stock cultures and placed in one of six per capita food treatments (1, 6, 11, 15, and 21 grains of powdered yeast/mite/d). Five replicates of each treatment were examined. Eggs were allowed to hatch, and the juveniles were monitored daily to maintain moisture conditions and levels of constant per capita food. The age at maturity and size at maturity were recorded for each individual within a tube. Size was measured as mite length using a graticule in the microscope eyepiece. We measured all mites under a standard magnification. A linear mixed effects model was used with tube as a random effect.

Our examination of egg size and number began by plac-

ing stock eggs in plaster-based tubes and rearing subsequent juveniles under high and low food conditions to generate two rearing conditions. At maturity, males and females were paired within treatments. Each pair was then assigned to either good (high food) or bad (low food) conditions. This created a fully factorial 2 (rearing) × 2 (current) design. There were 10 replicate pairs per treatment. Each pair was allowed to reproduce for 4 d. On day 4, adult pairs were transferred to new tubes and fed. The eggs laid between days 4 and 5 were then counted, and a sample of 10 eggs from each pair was measured lengthwise using an eyepiece graticule.

The relationship between egg size and adult size was assessed in an experiment that examined the fate of 60 eggs. Eggs were collected from the stock cultures, measured lengthwise, and reared on one ball of yeast/day. On maturity, adults were classified by sex and measured lengthwise.

The relationship between adult size and fecundity was assessed by relating the fecundity of females on the fifth day after maturity to the cube of female body length at maturity as an index of volume. Forty pairs of adults were reared from stock juveniles, and the pairs were fed daily throughout reproduction. The variability in these data sets arises from food treatments applied to juveniles and adults associated with other studies, but that generated a range of adult body sizes.

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One generation plants the trees; another gets the shade* – on maternal environments and offspring quality

Andrew P. Beckerman[1], Tim G. Benton[1], Craig T. Lapsley[1], Nils Koesters[1]

[1]Institute of Biological Science
University of Stirling
Stirling FK9 4LA
United Kingdom

Contact: Andrew Beckerman, IBIS, University of Stirling, Stirling FK9 4LA, UK;
apb1@stir.ac.uk;+44 178 646 7808;+44 178 646 4994(fax)

**chinese proverb*

In order to predict such events as extinction risk (Heino et al. 1997; Palmqvist and Lundberg 1998; Vucetich et al. 2000), the patterns in epidemics (Finkenstadt et al. 1998; Rohani et al. 1999), or harvest yield from natural resources (Hastings and Testa 1998), we need to know how varying environments are "filtered" by behaviour, life history and physiology into the population dynamics of an organism (Laakso et al. 2001; Ranta et al. 2000). Yet mapping the environment to population dynamics is not straightforward. An organism's life history is clearly a product of its age structure and its current environment. But a life history is also a product of an organisms' own history of environmental experience and, through parental effects, the environment of its ancestors (Roff 1992). This environmental history can generate delayed life history effects. Within a generation, these effects can lead to demographic patterns such as cohort effects. Maternal or paternal effects occur when delayed life history effects cross generations. Thus, in a variable environment, the conditions at one time can influence life history traits and performance of individuals at future times and subsequently, their population dynamics (Beckerman et al. 2002b; Ginzburg and Taneyhill 1994; Inchausti and Ginzburg 1998; Lindstrom and Kokko 2002).

Interest in maternal effects has been driven to a large extent, by research on the egg size or quality vs egg number tradeoff. Many studies have demonstrated that parental environments can alter resource allocation decisions by parents for offspring that lead to differential offspring quality (Mosseau and Fox 1998; Mousseau and Fox 1998; Rossiter 1998). More recently, studies have begun to document how parental environments, and these maternally transmitted delayed life history effects, can alter juvenile performance and fitness after allocation decisions have been made (LaMontagne and McCauley 2001; Mousseau and Fox 1998; Sinervo and Doughty 1996) or in the context of trophic interactions (Fox 1994; Fox 1997; Fox and Savalli 2000). Moreover, recent population dynamics studies have demonstrated that maternal effects and more generally delayed life history effects can have

significant effects on population dynamics (Ginzburg 1998; Inchausti and Ginzburg 1998; Lindstrom and Kokko 2002). The common lesson from all of these evolutionary, life historical and population dynamic studies is that the interactions between environmental variability and life history can occur both within and between generations (Bjornstad et al. 1998; Coulson et al. 2001; Dennis et al. 2001; Ginzburg 1998; Ginzburg and Taneyhill 1994; Inchausti and Ginzburg 1998; Stenseth et al. 1999). We may expect then that (st)age structure, the life cycle and the sequence of environmental variability will be necessary ingredients in explanations of population dynamics.

How can parental environments affect offspring performance?

Delayed life history effects that have crossed generations are clearly a product of the tradeoff between offspring size or quality and offspring number. While this tradeoff encompasses both the quality and the quantity of offspring, each of these measures can influence the performance of offspring in different ways. The "classic" maternal effect is based on a genotype x environment interaction affecting the transmission of resources, and thus "quality", from parent to offspring (Mousseau and Fox 1998; Rossiter 1996; Rossiter 1998). The maternal environment, acting on the tradeoff parents face between offspring quality and quantity, can alter resource allocation to individual offspring and can thus affect the performance of those offspring later in their lives. However, the parental environment also affects parental fecundity. While linked to allocation patterns by the tradeoff, fecundity patterns determine the competitive environment that offspring face and as a result, offspring performance in the future. For example, if parental environments reduce average fecundity, juvenile population density is subsequently reduced, possibly generating a favorable environment for juvenile growth and development.

The defining characteristic of the quality route is that patterns of maternal investment influence individual offspring quality directly. Rossiter (1996; 1998) has examined a wide

variety of routes by which the environment acts on allocation process and pattern, extending a historically genetic definition to one centred on genotype x environment interactions. Even with this broader definition, a core requirement remains that there must be maternal investment in individual offspring quality linked to environmental history (Mousseau and Fox 1998; Rossiter 1998). The defining characterisitic of the quantity route is that fecundity, and the numerical changes in offspring density that it represents, alters the competitive environment that offspring face. The performance consequences of this route are not driven directly by maternal investment in individuals, but by the number of individuals produced. The delayed life history response arises as an emergent, density dependent, population level property of adult performance in the parental environment.

In this paper we provide experimental evidence that parental environments can influence offspring performance by both a "quality" and "quantity" route. We experimentally evaluated the effects of variation in the parental environment on the hatching, growth and recruitment of the offspring generation using a model system of the soil mite *Sancassania berlesei*. We manipulated food and density during the development and the expression of traits in the parental generation as well as the development and expression of traits in the offspring generation, leading again to adulthood. We statistically partitioned the relative importance of parental versus juvenile environments to the life history and performance of offspring, paying special notice to the "quality" and "quantity" routes by which maternal environments can affect offspring performance. By manipulating food and density, we are, in effect, examining the interaction between maternal environment and offspring environment (e.g. Rossiter 1998) on density dependent life history traits. The design allows us to answer the question: by what routes do parental environments influence offspring performance?

Effects of parental environments on offspring performance: pattern and process

Our data are collected from a longitudinal laboratory experiment that covers almost two generations (see Appendix 1 for full design and analysis details, Beckerman et al. 2002a). It is a factorial experiment that varies food and density in a controlled manner throughout a lifecycle and one in which we measured many life history traits. To begin the experiment, eggs from second generation mothers reared from stock cultures were assigned to two PARENTAL REARING treatments (good or bad). Upon maturation to adulthood, males and females were paired randomly in three PARENTAL DENSITIES (1, 20, 50 pair) and then assigned randomly to two PARENTAL FOOD AMOUNTS (low and high) and two PARENTAL FOOD TIMINGS (no delay or a 5 day delay in receiving food after maturation). Eggs from these parents were then collected at two MATERNAL AGES (young and old) and the offspring from these eggs subject to a range of OFFSPRING treatments. Newly hatched offspring were subject to the range of JUVENILE DENSITIES that they were born into, and subsequently assigned randomly to high and low JUVENILE FOOD AMOUNTS that were delivered with two JUVENILE FOOD TIMINGS throughout their development (overtime or in one pulse at hatching). This factorial design allows a description of how variation in the maternal environment interacts with variation in the current offspring environment to shape offspring performance. By treating groups of mites as cohorts and examining vital rates under a range of current and historical treatments, we can develop an understanding of how life history plasticity can lead to delayed life history effects and how these delays can arise across generations.

Quantity vs. quality driven effects

The crux of our analysis lies in the statistical separation of maternal environment effects from offspring environment effects and the subsequent identification of quantity vs. quality routes. The maternal environment was characterized by PARENTAL REARING CONDITIONS,

PARENTAL DENSITY, PARENTAL FOOD AMOUNTS, PARENTAL FEEDING TIME, and MATERNAL AGE. The offspring environment was characterised by JUVENILE DENSITY, JUVENILE FOOD AMOUNT, and JUVENILE FEEDING TIME. We examined the influence of the "quantity route" on the percentage of juveniles recruiting, the age-at-maturity and size-at-maturity by statistically examining the effects of the above treatments. In particular, we were interested in juvenile perfomance patterns when parental environments altered fecundity and thus the initial density (competive environment) that offspring experience.

Detecting quality driven effects was slightly more involved. In our constant temperature experiments, hatching time is only defined by patterns of maternal allocation to eggs. The patterns of egg hatching can thus be attributed to the interaction between parental environments and maternal investment in egg quality. On the other hand, the juveniles' recruitment rate, their age-at-maturity, and their size-at-maturity are density dependent (juvenile density, see Beckerman et al. 2002a) and are likely to be affected by both the maternal investment in egg/offspring quality and the patterns of parental fecundity that can alter the competitive environment that offspring experience.

In order to detect the effects of allocation decisions in these life history traits that are not a consequence of the responses to density, we examined recruitment rates, age-at-maturity and size-at-maturity for individuals whose parents experienced poor (bad rearing, low food, five day delay) versus good conditions. Data from experiments on the tradeoff between egg quality and egg number in these mites (Benton et al, MS) indicate that parents reared under poor conditions produced fewer eggs of high quality than those reared under good conditions. Subsequently, the higher offspring quality may lead to higher recruitment rates, an earlier age-at-maturity and a larger size-at-maturity. We examined patterns in

recruitment, age-at-maturity and size-at-maturity in our data set for these quality derived effects.

Results: Time-to-hatching

We examined the time-to-hatching using survival analysis having followed the fate of all eggs laid. A Weibull distribution best fit the data (see appendix 1 for distribution selection methods). Our minimum adequate model for hatching time contained six significant two-way interactions (all $8 < \chi^2 < 2140$, df=1 or 2, all P<0.003). Interactions featuring PARENTAL REARING conditions and current adult conditions explained a substantial amount of hatch time variability. For example, well fed parents (PARENTAL FOOD) produced eggs that hatch around day four, independent of the rearing conditions (PARENTAL REARING) that those parents experienced during their development (Fig. 1a). However, for poorly fed parents, all eggs hatch earlier, but those parents reared under poor conditions laid eggs that hatched after ~three and one half days vs. well fed parents whose eggs hatch around day three (Fig 1b; $\chi^2_{\text{rearing x food}} = 908.65$, df=1, P<0.001). A similar interaction was detected between PARENTAL REARING and PARENTAL DENSITY treatments where hatching times for good or poorly reared adults were not different at low densities, but differed by nearly a day at moderate and high densities ($\chi^2_{\text{rearing x density}} = 117.20$, df=2, P<0.001). These interactions suggest that the time-to-hatching is sensitive not only to the conditions mothers face as adults, but also to the conditions mothers experienced as juveniles growing up.

Mothers' age, interacting with the amount of parental food, explained the largest amount of variance in hatching time (Fig 2a,b; $\chi^2_{\text{food x maternal age}} = 2149.86$, df=1,P<0.001). Mothers' age may be a clear indicator of quality driven effects in time to hatching as there are well known changes in provisioning and fitness with age (Benton et al. MS; Fox et al. 2001;

Roff 1992; Stearns 1992). In our study, older mothers produced eggs with a median hatching time of approximately three and a half days, independent of food amounts. Younger mothers, however, produced eggs with median hatching times that varied from three and a half days with restricted food to four and a half days with large amounts of food. Thus, food levels influenced egg development when mothers were young but not when mothers were old. Our data indicate that the environment that mothers experience as adults and during their development as juveniles can alter the timing of their offsprings' hatching. As hatching is not density dependent, these changes can be attributed primarily to allocation decisions made by mothers in response to their environment and relative to their age.

Results: Proportion Recruiting

We analysed the proportion of juveniles recruiting (proportion of eggs laid becoming adult, irrespective of age-at-maturity) using a generalized linear model with binomial errors. We used F-tests rather than Chi-square tests to account for overdispersion in the binomial data (see appendix 1). As with hatching time, our minimum adequate model contained a wide range of significant two-way interactions. Controlling for age-at-maturity ($F_{age\text{-}at\text{-}maturity}$ = 16.57, df=1, P<0.001), the largest amount of variability in recruitment was explained by the interaction between PARENTAL REARING conditions and the delay in food provision to parents (PARENTAL FOOD DELAY; Fig 3; $F_{rear \times delay}$ = 30.77, df=1, P<0.001). When food was delivered at the start of adulthood, recruitment rates averaged 60% and were unaffected by rearing conditions. However, delaying feeding for five days after maturity resulted in a marked overall increase in recruitment and a sensitivity to adult rearing conditions. When parental food was delayed, offspring recruitment was just over 85% if parents were reared well and nearly 95% when they were reared poorly.

This is clear evidence that the quantity route between maternal environments and offspring performance can be strong. Poor adult rearing and a delay in adult feeding are two

significant factors affecting adult fecundity (Beckerman et al. 2002a). These two treatments cause significant reductions in fecundity, leading to lowered offspring population densities. As recruitment to adulthood is density dependent and linked to juvenile food amounts (Beckerman et al. 2002a), this shows that the maternal environment can alter offspring performance without classical maternal effects, and independent of juvenile conditions. These data show that it is possible for a quantitative life history attribute of mothers (fecundity) to significantly alter the competitive environment of juveniles and thus affect juvenile performance.

Interactions between parental and juvenile environmental conditions were also common. PARENTAL REARING ($F_{\text{parental rearing x juvenile food}} = 9.38$, df=3, $P<0.001$), PARENTAL DENSITY ($F_{\text{parental density x juvenile food}} = 3.24$, df=6, $P=0.005$) and the PARENTAL FOOD DELAY ($F_{\text{parental delay x juvenile food}} = 5.72$, df=3, $P=0.001$) all predictably influenced the effect of juvenile food on recruitment rates. Juveniles fed over time always showed higher recruitment. However, as above, when parents experienced a delay in receiving their first food, recruitment was increased. Increasing adult density only reduced recruitment when food for juveniles was restricted to a single pulse of food. Finally, good adult rearing tended to increase recruitment, but only when food was restricted during juvenile development.

The results above have highlighted the quantity route by which maternal environments can influence recruitment rates in the subsequent generation. The dependence of fecundity on the parental environment can dramatically alter juvenile population density, leading to cohorts of offspring that perform differently from each other. However, controlling for these effects, our analysis also showed that maternal age and the delay in parental feeding explain the second largest amount of variance in recruitment (Fig 4; $F_{\text{maternal age x adult delay}} = 21.07$, df=1, $P<0.001$). When there is no delay in feeding, recruitment rates for

offspring from young or old mothers are indistinguishable. However, when a five day delay in parental feeding was imposed, young mothers were severely affected by food restrictions, leading to lower fecundity and subsequently higher offspring recruitment. While this may be considered evidence of maternal investment patterns, our experimental design allows this pattern to arise as a quantity effect. Eggs from young mothers were gathered on days four and five after maturity, meaning that young mothers experiencing a delay had no food to lay their initial batch of eggs. Not surprisingly, these were very low numbers and as a result, this effect also represents a demographic effect of the maternal environment.

In order to detect the effects of the quality route, we examined the performance of offspring from parents that have experience good conditions during development and throughout their lives vs. parents who have experienced the opposite (see *Quantity vs quality driven effects* above) . We first classified individuals as coming from good or bad backgrounds. Good backgrounds were defined by good rearing, high food and no delay in feeding, while poor backgrounds were defined by bad rearing, low food and a five day delay in feeding. A binomial regression on recruitment as a function of parental background, controlling for adult density, juvenile density and juvenile food produced a pattern in recruitment consistent with our hypothesis: assuming that individuals from a bad background produce a few, high quality eggs, the offspring from these eggs had higher recruitment rates, independent of the effects generated by the competitive environment juveniles face (bad background: 63.2% versus good background: 36.9%; $F_{background} = 39.5708$, df=1, $P<0.001$ $F_{adult\ density}=7.15$,df=1, $p<0.05$, $F_{juvenile\ food} = 54.8986$, df=3, $p<0.001$, $F_{juvenile\ density}$ 23.4880 df=1, $P<0.001$).

Results: Age-at-maturity

We analysed age-at-maturity using survival analysis, having followed the development of individuals from hatching to maturity. Our data were best fit by a Weibull distribution. Our

minimum adequate model was fully specified at the two - way interaction level. Chi-square values for the interactions ranged from 5 to 1900. The highest values ($\chi^2 > 900$) correspond to interactions between PARENTAL DENSITY and JUVENIEL DENSITY ($\chi^2 = 1182$, df=2, $p<0.001$), PARENTAL DENSITY and JUVENILE FOOD ($\chi^2 = 1166$, df=6, $p<0.001$), MATERNAL AGE and JUVENILE FOOD ($\chi^2 = 1080$, df=3, $p<0.001$), PARENTAL FOOD DELAY and JUVENILE FOOD ($\chi^2 = 911.20$, df =3 , $P<0.001$) and JUVENILE FOOD and JUVENILE DENSITY ($\chi^2 = 1961$, df=3, $p<0.001$).

For example, increasing juvenile density leads to a later age-at-maturity (compare Figs. 5a and 5c). This is consistent with previous data on the effects of plasticity in juvenile performance (Beckerman et al. 2002a). Accounting for adult density (Fig 5a-c) indicates that the lengthening of the juvenile life span by juvenile density can be contingent on the density their parents experienced as adults. Compared to low adult densities, medium and high adult densities lead to proportionately later maturity for a given juvenile density. Higher adult densities lead to higher offspring densities in our experiments (though per capita fecundity decreases) maintaining the consistent pattern that age-at-maturity increases with increasing density, but via a nonlinear interaction between adult fecundity and juvenile density.

Also in line with previous analyses (Beckerman et al. 2002a), we found that reductions in JUVNENILE FOOD (Pulsed vs. Overtime feeding for juveniles) lengthened the juvenile stage. However, we also found that an ADULT FEEDING DELAY causes an increase in age at maturity (Fig. 6). This is interesting because a five day delay in parental feeding tends to lower juvenile density and lower juvenile density tends to favor an earlier age at maturity (Fig 5). But, it appears that if your parents were not fed until five days after they matured, juveniles would mature later for a given amount of food, and much later if they were under poor food conditions. This may reflect the parents' needing to allocate more provisions to themselves after a period without food, thereby reducing allocation to the eggs.

Experimental data (A.P. Beckerman, T.G. Benton, *unpublished data*) suggests that under low food conditions, such as occurs when a "pulse" of food has been consumed, individuals can survive for up to a 100 days following the last feeding and mature at this point. This is, at least in part, because individuals that die are consumed, so there is some recycling of nutrients. Under such low food conditions, the size of an individual may be an important determinant of competitive ability, and so the positive "headstarting" given by a well-provisioned egg may become particularly important. Under conditions when food is plentiful, the importance of the parental provisioning of the egg may matter less. Thus, as has been recently found with other tradeoffs (such as the costs of immune defence are recognisable only in resource-challenged individuals, Moret and Schmid-Hempel 2000), the costs and benefits of life-history decsisions may be context dependent. Thus, studies which conclude that maternal effects are not biologically important may draw this conclusion because the experimental organisms have experienced conditions which do not allow the effects to be distinguished.

There is a challenge in visualizing the range of significant effects our fully parameterized model of age-at-maturity where all terms are significant. One method for visualizing the results of complex designs is to perform a regression tree analysis on the predicted values from a model (F.A.Harrell, personal communication, S/R-News user group). When applied to the predicted or fitted values of a model, this becomes a strategy of visualising the way that variance in the predictions can be apportioned to different factors and covariates. Figure 7 presents our statistical results as a regression tree. Used this way, a regression tree analysis is not a rigorous analytic tool, but a way of visualising the importance of different factors and covariates in our model of age-at-maturity. Regression trees describe the structure of data by iteratively splitting the data into homogenous groups defined by the factors and covariates in an experiment. Each homogenous group explains an amount of

variance or deviance at that point in the heirarchy and the same factor can split a node at different levels in the hierarchy. It is therefore possible to "prune" a tree to an R^2 of 95% to classify the structure of the data based on a set of factors. Our tree analysis employed the rpart library for R (Therneau and Atkinson 2000).

Clearly, the regression tree and the Chi-square values tell a similar story. Age-at-maturity is largely determined by the effects of juvenile food and juvenile density. However, we can see that the effects of the parental delay in feeding, adult density and maternal age all figure in the explanation of age-at maturity. After juvenile food and juvenile density split the variation, the predominantly numerical (quantity) effects of the delay in parental feeding figure strongly and seem to govern the distribution of the very late maturity times (see above for details). As with the effects of parental environment on recruitment, these data suggest that parental environments and parental density dependent life history can interact with juvenile conditions to shape juvenile performance.

Detecting and describing the effects of maternal investment in quality that are independent from responses to adjustiments of the competitive environment by parents requires again that we examine the performance of offspring from mothers that had good backgrounds and mothers that had poor backgrounds, controlling for adult density, maternal age, juvenile density and, considering our result above, juvenile food. Our premise was that mothers from poor backgrounds invest more per egg and in fewer eggs leading to eggs that would hatch earlier. Following a similar protocol to that for recruitment, we performed a survival analysis to determine the age at maturity of offspring from parents that experienced good and bad conditions. This model suggests that there can be a 20 day reduction in median age-at-maturity between the two background conditions, controlling for the effects of current juvenile conditions (bad background: 17.6 days versus good background: 36.9 days; $\chi^2_{\text{background}} = 19.49$, df=1, $P<0.001$ $\chi^2_{\text{adult density}}=332.8$, df=1, $p<0.001$,$\chi^2_{\text{juvenile food}} = 16318.92$,

df=3, $p<0.001$, $\chi^2_{\text{juvenile density}}$ 3318.22, df=1, $P<0.001$, $\chi^2_{\text{maternal age}} = 99.82$, df=1, $P<0.001$). Thus, our data suggest a pattern in age-at-maturity that is consistent with our hypothesis that maternal investment can influence the growth and development of juveniles independent of the treatments and conditions that can alter the juvenile competitive environment

And based on the interaction between juvenile food and the delay in parental density relative to the more general analysis of good and bad backgrounds, variability in juvenile food may alter the way in which investment by mothers into offspring is expressed. This suggests a context dependence in "quality" driven effects. Our data indicates that there may be juvenile environmental conditions that when combined with maternally driven increases in egg quality and maternally driven differences in the juvenile competitive environment, lead to substantially shorter or longer development times. Thus, field and lab studies that fails to demonstrate the influence of maternal environments on juvenile performance are likely correct , but the generality of these conclusions must be framed in the context of the patterns of environmental variance that the organism might experience (e.g. Ergon et al. 2001; Moret and Schmid-Hempel 2000).

Results: Size at Maturity

We analyzed size-at-maturity with a generalized linear model with gaussian errors. Our data on size are quite sparse relative to the other traits, and as a result we limited our assessment of size to the question of quality effects. Our best model controlled for recruitment levels ($F_{\text{recruit}} = 11.90$, df=1, P=0.002), PARENTAL FOOD ($F_{\text{parental food}}=7.49$, df= 1, p= 0.011), MATERNAL AGE ($F_{\text{maternal age}}= 21.69$, df=1, $P<0.001$), JUVENILE DENSITY and JUVENILE FOOD ($F_{\text{juvenile density x juvenile food}} = 5.93$, df=3, p=0.0034) and found a significant interaction between PARENTAL REARING and PARENTAL DELAY ($F_{\text{parental rearing x parental delay}} = 4.06$, df=1, p=0.05) in feeding. We found (Fig. 8) that offspring from parents that were reared poorly as juveniles were larger when their parents faced a delay in

feeding but smaller when their parents did not face this delay. If our assumption is correct, then parents facing bad conditions as juveniles and as adults will invest more in fewer eggs leading to comparitively larger offspring than when conditions are not so poor for the parents.

The route from mother to offspring

We proposed that there are two routes by which maternal environments can influence the performance of offspring. In the first route, the response of parental fecundity to the adult environment has a "quantity" effect on the population density of juveniles and thus their competitive environment. This is not a maternal effect per se, because the route to these delayed life history effects are not driven by patterns of maternal investment in offspring quality, even though fecundity is part of the egg size/quality – egg number tradeoff. This "quantity" route can lead to the formation of cohort effects in offspring life stage via parental life history responses to maternal environments. In the second route, the maternal environment affects maternal allocation of energy among survival, current and future reproduction and egg size/quality and egg number. The ability for parents to invest in the quality of eggs results in differential offspring performance leading to the classical definition of a maternal effect.

We have provided evidence that both of these pathways can be important in these soil mites. Patterns in time to hatching, being independent of juvenile density (and hence adult fecundity) and juvenile food, show that maternal environments likely lead to differential maternal investment in eggs. The effects of variable parental food and density, as well as the parental rearing conditions lead to quite variable hatching times. Percent recruitment and age-at-maturity show evidence for both routes. The "quantity" route is driven primarily by plasticity in fecundity arising from a delay in parental feeding but also by variability in parental density, mothers age and parental rearing conditions. Thus, there are detectable effects in the performance of juveniles caused by the conditions their parents experienced as

juveniles, a generation in the past. The experimental conditions are similar to the type of variation that might arise in real world environments and our treatments generated significant density dependent responses in offspring performance through lowered or raised offspring density. These density differences can cause large increases in recruitment rate and, under good conditions, a reduction in the age-at-maturity. These patterns illustrate that plasticity in parental life history traits can influence the competitive environment that offspring face and thus their performance.

Our evidence for allocation effects came from statistical evaluation of the premise that adults that experienced poor conditions will produce fewer, higher quality eggs and that these higher quality eggs will possess the resources to induce a higher probability of maturing, an earlier age-at-maturity and a larger size-at-maturity. Our assessment of recruitment patterns revealed that there could be a 20% improvement in maturation success when parents experienced extreme bad backgrounds and also up to a 20 day decrease in age-at-maturity under those same conditions. Moreover, these same bad conditions can increase size-at-maturity as well. The evolutionary argument for these patterns is straightforward: in temporally autocorrelated environments, parents that experience poor conditions are more likely to invest in offspring in a manner that will allow those offspring to handle similar poor environments (e.g. Sinervo et al. 2000). Our experimental design happened to highlight what can happen when these better provisioned offspring also experience relatively good conditions.

Egg quality-egg number tradeoff: a route for transgeneration delayed life history effects

Our data thus support the premise that the parental environment can influence offspring performance though the "quality" and the "quantity" route. We have in essence proposed that the egg quality – egg number tradeoff is central to the formation of delayed life history effects that are expressed in offspring but originate in parental generations. The parental

environment directly influences fecundity and as such can determine the density dependent, competitive environment that juveniles experience. Combined with food availability for juveniles, there are clear, fecundity driven links between parent and offspring that define the "quantity" route. Moreover, the fecundity response is not simply numerical because it is linked to the tradeoff between number and quality of offspring. The density dependent responses of parents are intimately linked to allocation patterns such that egg quality variation may contribute substantially to the patterns of juvenile performance – the "quality" route. The fitness consequences of these allocation driven relationships can be substantial. Egg size and quality is positively related to female and male size at emergence ($F_{2,62} = 139.3$, $P<0.001$, $R^2=0.82$) as well as to the timing of hatching and maturity. Larger females also go on to produced more eggs as measured by peak fecundity ($fecundity_{day\ 5\ after\ maturity} = -33.25+0.0044$ x female volume; $F_{1,55} = 48.49$, $P<0.001$, $R^2 = 0.47$), making it clear that quality and quantity are two distinct but interlinked routes connecting parental environments to offspring performance.

Summary

Identifying patterns of density dependence in life history traits has been critical to the advancement of ecological research and to the improved understanding of the processes underlying population dynamics. Our data highlight that substantial variability in density dependent life history traits can arise both as a function of current environments, and as a function of historical environments – in our case, parental environments (see also Beckerman et al. 2002a). Such parental and maternal effects have been studied for quite some time, primarily centred around the consequences of allocation decisions between offspring size/quality and number (Fox et al. 2001; Hendry et al. 2001). Our data add to a small but growing body of experimental evidence that maternal effects on offspring quality can have profound and detectable effects on performance characteristics of juveniles throughout their

lives (LaMontagne and McCauley 2001; Mousseau and Fox 1998; Sinervo and Doughty 1996).

Moreover, our data show that the route by which the maternal environment can alter offspring performance is at least twofold. Rossiter (1998) expanded the range of routes by which classic maternal effects can arise by focusing on the array of genotype x environment interactions that can exist between parent genotype, parent environment, offspring environment and offspring performance leading to variation in investment to individual offspring. Our experiments highlight an alternative, but complementary route that reinforces recent conclusions from population dynamic studies about the importance of delayed life history effects and age structure (Bjornstad et al. 1999; Coulson et al. 2000; Stenseth et al. 1999). Our data show that environmental variability in parental generations can lead to variability in parental fecundity that subsequently alters the competitive environment that offspring experience. When this happens, density dependent juvenile traits can be linked directly to parental environments. Delayed life history effect arise via a quantity and a quality route.

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We thank Ken Wilson, Stewart Plaistow, Mark McPeek and two anonymous reviewers for comments on previous drafts. This research is funded by NERC (UK) grant GR3/12452 to TGB. Alex Alardyce, John McAlaney and Gemma Truelove helped collect data on egg size and number, egg size and adult size data and size and age-at-maturity data.

Appendix 1. Experimental Design and Analysis

The life cycle of *S. berlesei* consists of five stages: eggs followed by a larval, protonymph, and tritonymph stage and then adulthood. Our experimental mite populations are maintained in glass tubes (20mm x 50mm) ¾ filled with plaster of Paris that is kept moist to maintain humidity and kept at a constant 24°C in unlit incubators with food supplied in the form of granulated yeast. The experimental design is a straightforward factorial expansion of four binary treatments, one three level treatment, one four level treatment and one covariate.

The experiment began with eggs drawn from long running stock cultures initiated in 1996. The eggs, and then juveniles, were allowed to develop in one of two PARENTAL REARING CONDITIONS: good conditions defined by low densities (~20 mites) and ad libitum food or bad conditions defined by high densities (~100 mites) and restricted food. When the mites reached maturity, adults were paired into three PARENTAL DENSITY TREATMENS (1 pair, 20 pair or 50 pair), each replicated eight times at the level of REARING. Four of each set of eight tubes were then assigned to one of two ADULT FOOD TREATMENTS (1 ball or 5 balls) and then two each of these to one of two ADULT STARVATION TREAMTMENTS (fed at pairing or five days after pairing). Thus there are two replicates of each of the treatment combinations.

Eggs laid by these parents were used in our offspring experiments. Eggs were collected at two maternal ages: YOUNG (days 4-6) and OLD (days 9-11). Eggs from days 6 and 11 were used to assess hatching times. The remaining four sets of eggs (days 4,5,9,10) comprising two replicates each were randomly assigned a JUVENILE DENSITY SCORE (maximum juvenile numbers hatching) and then to one of four JUVENILE FOOD TREAMTMENTS . Food was either pulsed at hatching or delivered over time throughout development and it was provided ad-libitum or in a limited manner, judged by density, leading to lo-pulse, hi-pulse, lo-overtime and hi-overtime treatments.

All data were collected by counting or measuring the different life stages under a binocular microscope (Leica, USA). All statistical analyses were implemented in R (Ihaka and Gentleman 1996) and use the HMISC and DESIGN libraries (Harrell 2000), the rpart library (Therneau and Atkinson 2000) and the MASS library (Venables and Ripley 1999). Our model selection criteria had the following structure for finding the minimum adequate model (Crawley 1993). Models for hatching and % recruitment were initially specified at the three-way interaction level. The age-at-maturity was specified at the two way level as the three way interactions were significant, but predictions nonsensical. AIC criteria were used to reduce the fully specified models. The use of AIC is effective for generating predictive models but can often retain effects that have p-values greater than 0.05. From this point on, we reduced the model by hand using p-values of 0.05 as a cut-off, thus generating a parsimonious, explanatory model.

Survival data (Time-to-hatching and Age-at-maturity) were analysed with parametric survival models (our data do not conform to Cox-proportional hazard assumptions). Selection of the appropriate distribution was based on an examination of censored model residuals and compared to theoretical null distributions (Harrell 2000). Recruitment data was analysed using a generalised linear model with binomial error structure. F-tests were used to assess significance because our data were over-dispersed. An F-test in a binomial model assumes a quasi-binomial family and is suitable for overdispersed binomial data.

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Figure Legends

Figure 1. Predicted survival curves showing the probability of egg hatching at time t. Panel A – Well fed mothers: the effect of parental rearing is non-existent. Panel B – Poorly fed mothers: all eggs hatch earlier and mothers reared well (good) produce eggs that hatch one half day earlier than poorly reared mothers (bad).

Figure 2. Predicted survival curves showing the probability of egg hatching at time t. Panel A – Young mothers: High food levels (ball) during reproductions result in later hatching. B – Old mothers: Food levels during reproduction do not affect hatching time, and median hatch time is similar to low food (grain) for young mothers.

Figure 3. Changes in percent recruitment (Mean ± SE) as predicted by the interaction between parental rearing conditions (Bad, Good) and the delay in feeding adults at maturity (none or 5 days). With no delay in feeding, good and bad rearing did not affect recruitment. Delaying adult food for five days after maturity greatly increased offspring recruitment and moreso for poorly reared parents.

Figure 4. Changes in percent recruitment (Mean ± SE) as predicted by interaction between maternal age (Young and Old) and the delay in feeding adults at maturity (none or 5 days). A five day delay in parental food increased the recruitment rate of offspring from young mothers but had no effect on older mothers.

Figure 5. Predicted survival curves documenting age-at-maturity patterns and the effect of the interaction between juvenile density (A: 250; B: 800; C: 1500) and adult density (2,40,100). As juvenile density increases age-at-maturity increases. As juvenile density increases (panel

A-C), the difference between adult density also increases. The vertical line references a 10 day age-at-maturity.

Figure 6. Predicted survival curves documenting age-at-maturity patterns and the effect of the interaction between juvenile food (lopul/hipul = low/high juvenile food in a pulse at hatching; loOT/hiOT = low/high juvenile food delivered over time from hatching) and the delay in parental feeding. In both panels, the effect of a reduction in juvenile food (pulse vs. over time) is to increase age at maturity. Moving from panel A to B shows how a five day delay in parental feeding increases the age at maturity for a given food level. The vertical line references a 10 day age at maturity.

Figure 7. The regression tree of predicted values from the fully parameterized, 2-way interaction survival analyses of age-at-maturity . The tree is pruned to 95%. Each branch specifies a homogenous split of variance based on the factor or covariate on the "branch" of the tree. In most cases only the right branch is labelled, the left being the opposite term. Bold labels correspond to characteristics of the parental generation. The terminal nodes present the actual predicted age-at-maturity.

Figure 8. Model fits of the interaction between parental rearing and parental delay in feeding on the size at maturity of offspring. Data are mean and standard errors of the model fits. They show that the effect of parental rearing on offspring size at maturity is dependendent on the conditions that parents face as adults.

Figure 1.

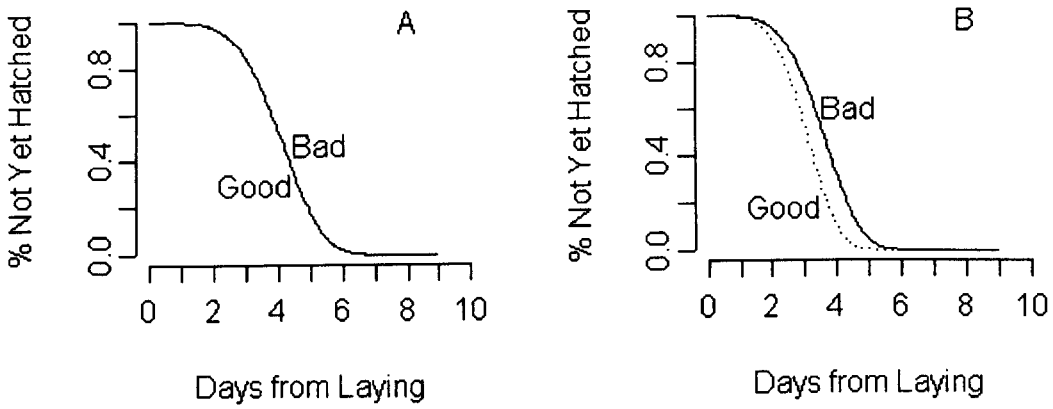

A
% Not Yet Hatched
0.0
0.4
0.8
Bad
Good
0
2
4
6
8
10
Days from Laying
B
% Not Yet Hatched
0.0
0.4
0.8
Bad
Good
0
2
4
6
8
10
Days from Laying

Figure 2.

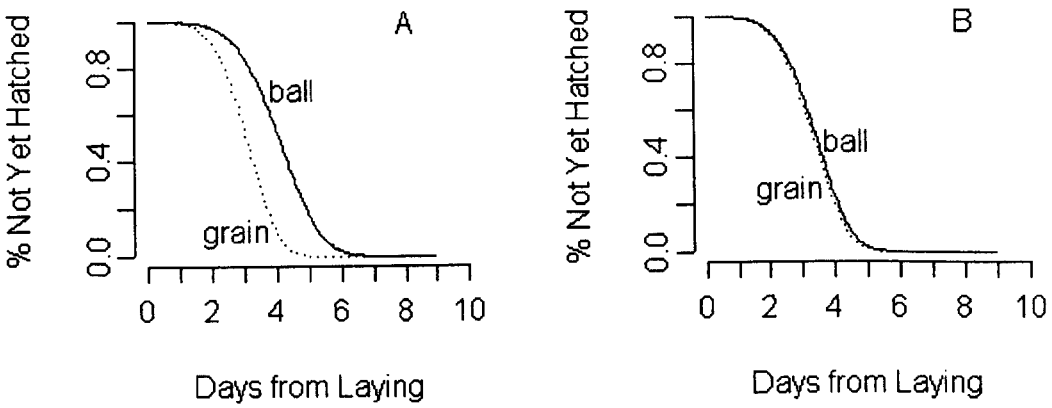

A
B
% Not Yet Hatched
0.0
0.4
0.8
ball
grain
0
2
4
6
8
10
Days from Laying

Figure 3.

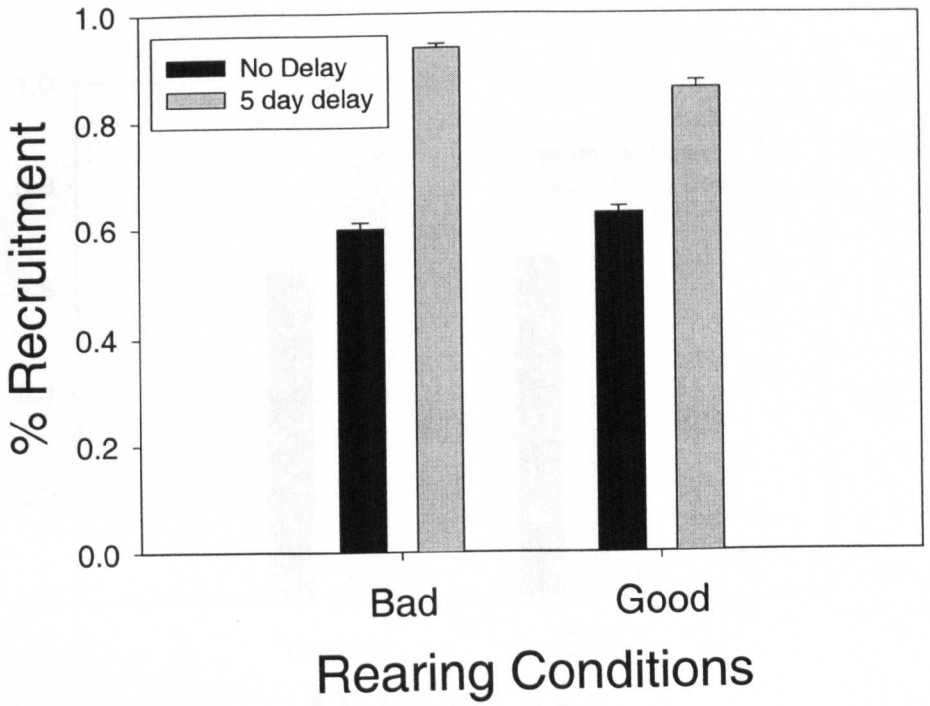

1.0
0.8
0.6
0.4
0.2
0.0
% Recruitment
No Delay
5 day delay
Bad
Good
Rearing Conditions

Figure 4.

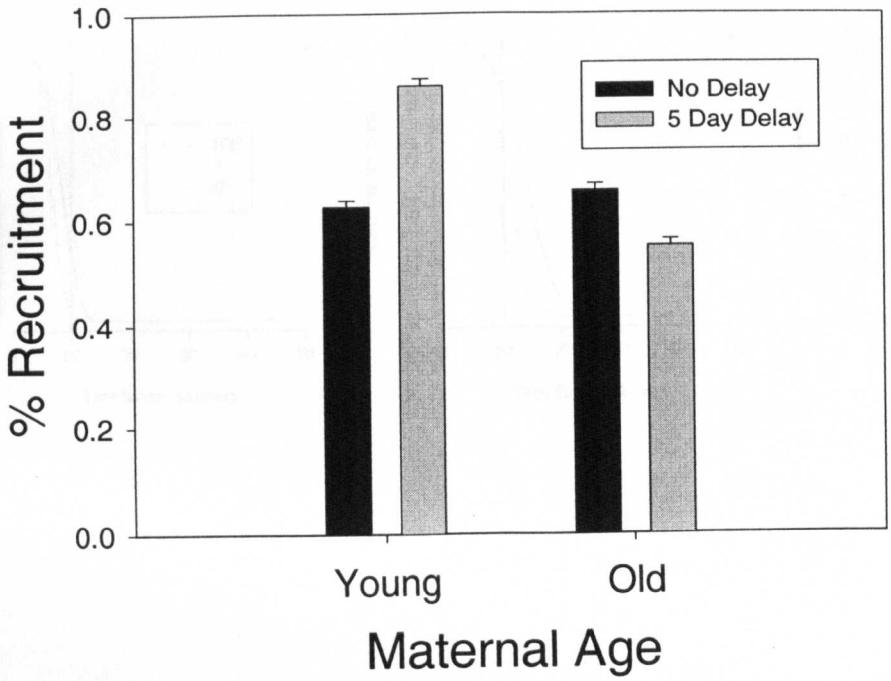

1.0
0.8
0.6
0.4
0.2
0.0
% Recruitment
No Delay
5 Day Delay
Young
Old
Maternal Age

Figure 5.

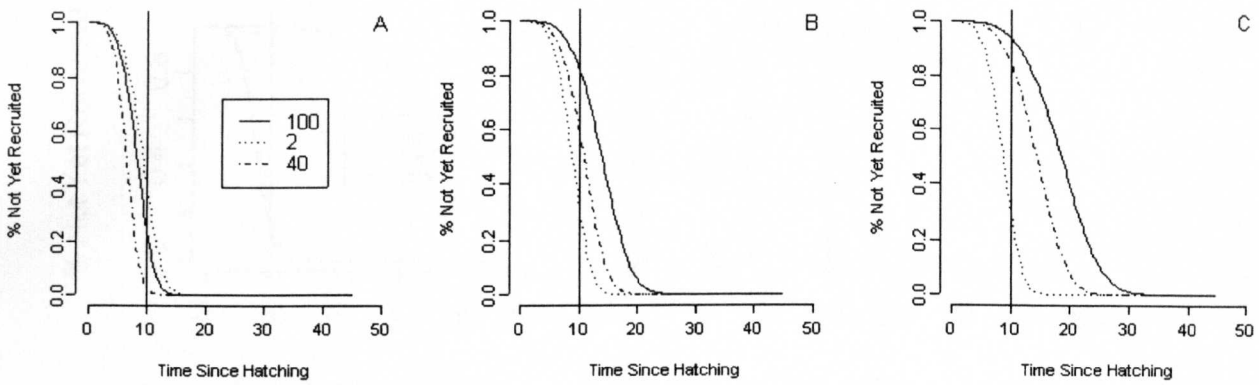

A
B
C
% Not Yet Recruited
Time Since Hatching
100
2
40
0.0
0.2
0.4
0.6
0.8
1.0
0
10
20
30
40
50

Figure 6.

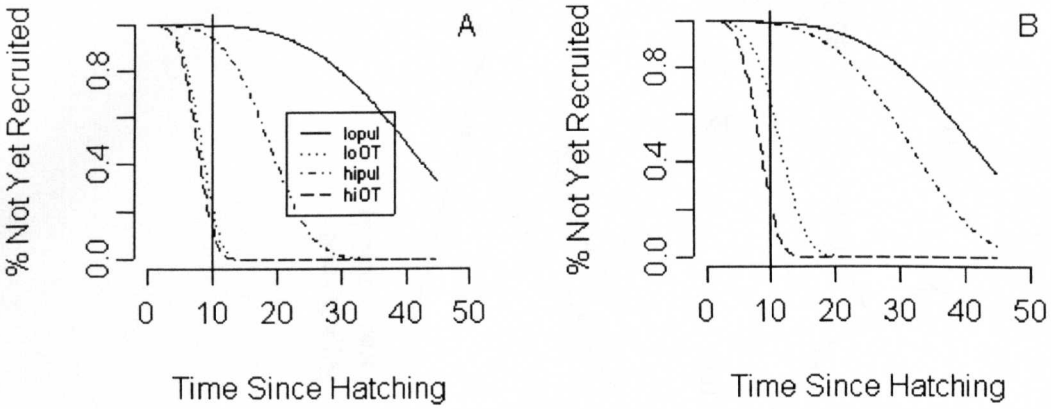

% Not Yet Recruited
0.0
0.4
0.8
A
lopul
loOT
hipul
hiOT
0
10
20
30
40
50
Time Since Hatching
% Not Yet Recruited
0.0
0.4
0.8
B
0
10
20
30
40
50
Time Since Hatching

Figure 7

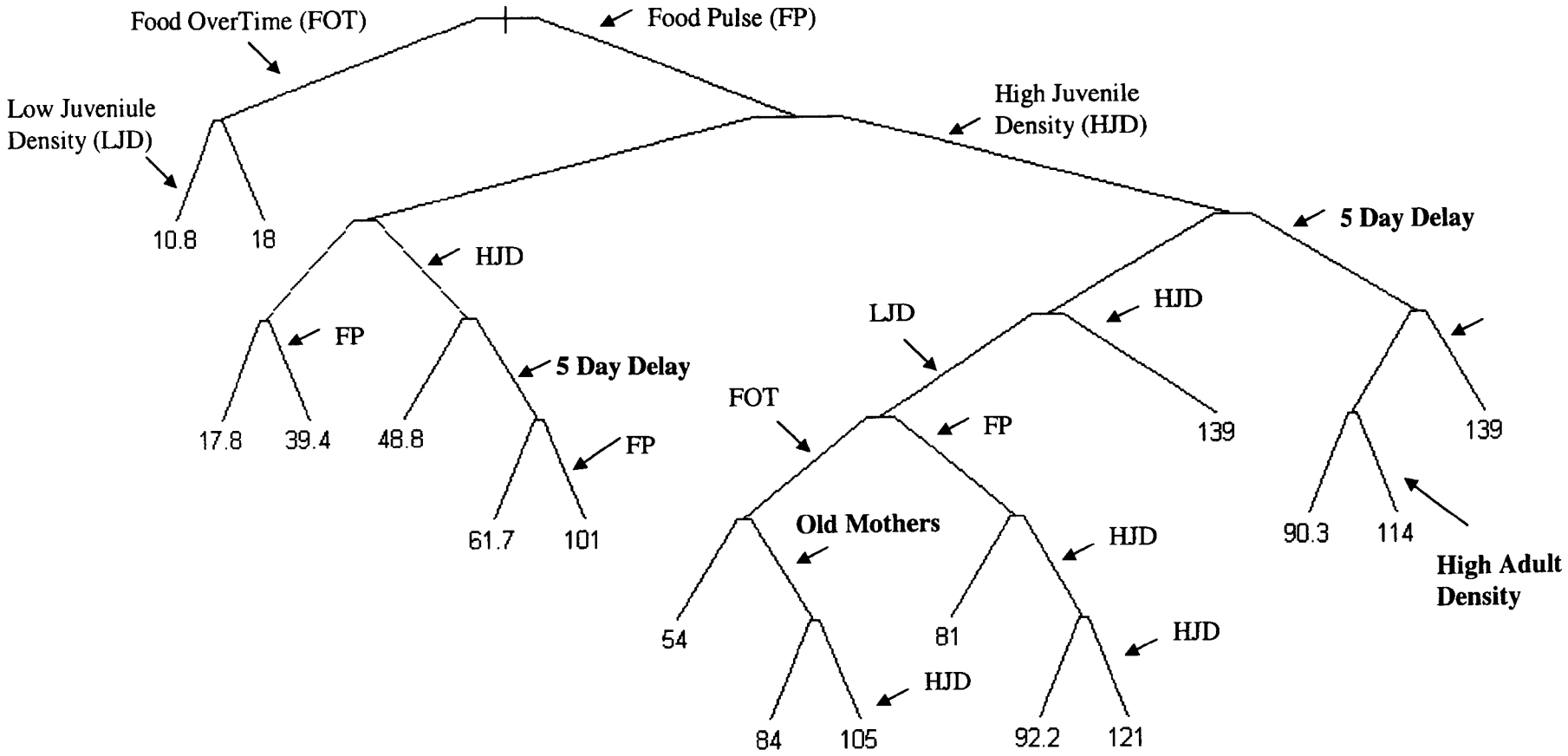
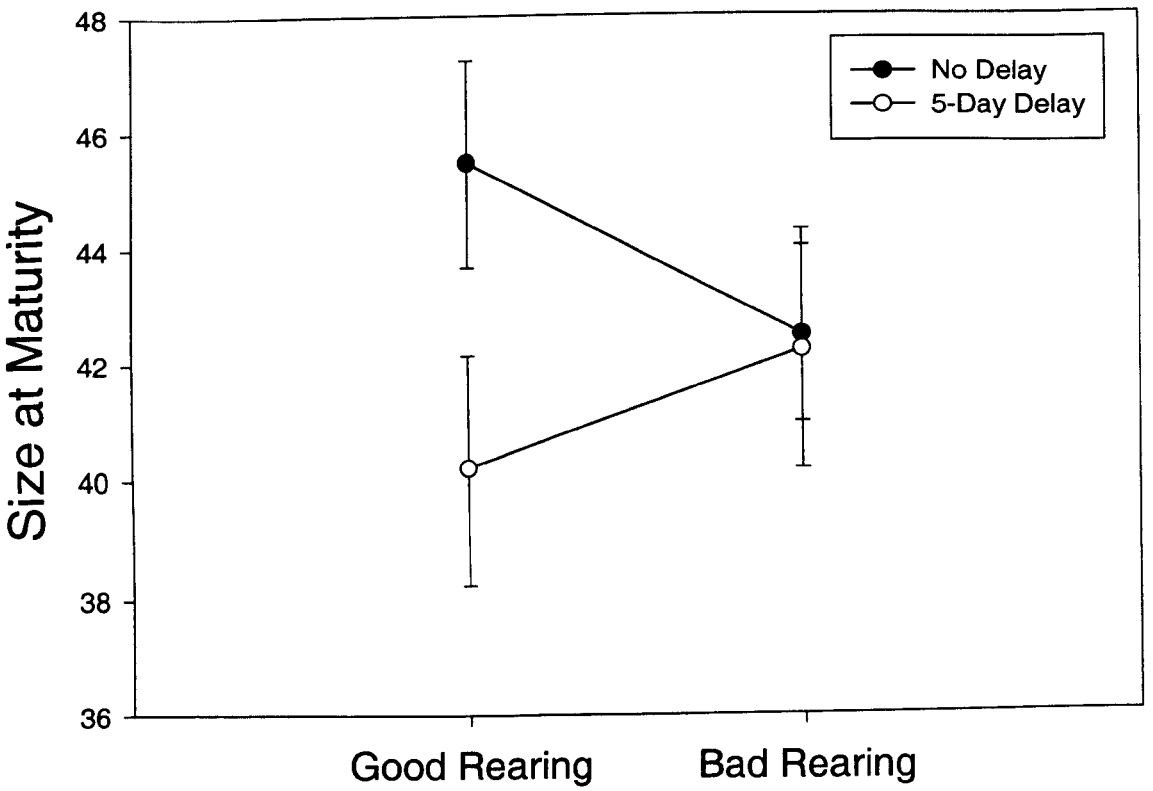

Food OverTime (FOT)
Food Pulse (FP)
Low Juveniule Density (LJD)
High Juvenile Density (HJD)
10.8
18
HJD
5 Day Delay
FP
5 Day Delay
17.8
39.4
48.8
FP
61.7
101
LJD
HJD
FOT
FP
139
139
Old Mothers
HJD
90.3
114
High Adult Density
54
81
HJD
HJD
84
105
92.2
121
Predicted Age-at-maturity

Figure 8.


48
46
44
42
40
38
36
Size at Maturity
No Delay
5-Day Delay
Good Rearing
Bad Rearing

Declaration

This thesis has been composed by me and the work it embodies has been conducted, unless otherwise stated, by myself and has not been included in another thesis.

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Nils B. Kösters