Evolutionary genetics of life-history traits in a haplodiploid mite

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Introduction, research questions and summary of results

This thesis has as its aim to better understand the genetic architecture of fitness traits in a small herbivorous arthropod, the two-spotted spider mite *Tetranychus urticae*. It provides a description and analysis of data on the amount and distribution of genetic variance within traits and the presence of genetic covariance between traits. This information is used to address some fundamental questions in the theory of life-history evolution. The questions are based on the premise that life-history traits are under strong directional selection and the subsequent theoretical expectations for the genetic architecture of these traits. Below an introduction is given to the theory of life-history evolution as far as is relevant to this thesis (*The evolutionary genetics of life-history traits*). Then I provide a brief description of the biology of *T. urticae* (*The two-spotted spider mite*). In order to analyze the typically polygenic traits of a life history I used quantitative genetics theory, the basics of which are discussed in Box 1.1. Finally, a description of the specific questions in this thesis is given, together with a short summary of the results obtained (*Questions and summary of results*).

The evolutionary genetics of life-history traits

Life-history traits are major components of fitness and involve an organism’s growth, reproduction and survival (Futuyma 2009). They include traits such as the rate of reproduction, maturity rate, juvenile survival and life span. Life-history traits are directly related to fitness; an increase in a life-history trait leads to an increase in fitness. This relationship makes these traits particularly interesting for a study of genetics and selection, because they are expected to be under strong directional selection. This leads to simple predictions regarding their genetic architecture (see Box 1.1 for an explanation of the genetic terms used). First, directional selection is expected to yield low levels of additive variance. Second, directional selection is expected to cause relatively high levels of directional dominance variance. Third, genetic trade-offs with other fitness traits are expected to impose limits on the evolutionary pathways of individual life-history traits. Below I sketch the context of these three expectations separately.
CHAPTER 1

Box 1.1

Quantitative genetics

Most traits are governed by a multitude of genes that have small individual effects. Quantitative genetics examines the genetic properties of the variation of these complex traits. It is a statistical branch of genetics that is based on fundamental Mendelian principles but extended to polygenic traits. Consequently, the level of investigation of quantitative genetics is populations, with formulations phrased in terms such as means and variances (Lynch and Walsh 1998). All parameters are based on phenotypic values; the value of a particular trait observed when measuring an individual. Differences between individuals of a population are quantified by variance, $V$. The total observed variance (phenotypic variance, $V_P$) in a population can be caused by environmental variance ($V_E$), genetic variance ($V_G$) and an interaction between these two (Falconer and Mackay 1996).

The genetic variance can be divided into three main subsections: additive ($V_A$), dominance ($V_D$) and epistatic ($V_I$) variance. Additive variance refers to that part of an individual’s genotypic value that is expressed in the phenotype of its offspring. Dominance and epistatic variance refer to the remaining unexpressed part of the genotypic value and are therefore of less relevance in research on inheritance in outbred populations. However, dominance variance is a central factor for fitness in situations of inbreeding, where increased homozygosity leads to recessive alleles being expressed (see the section on life-history evolution). Another important parameter in quantitative genetics is the narrow-sense heritability of a trait: $h^2 = V_A/V_P$. It is a measure for the degree to which phenotypes are determined by genes transmitted by the parents. A term for the non-additive variance is residual variance: $V_R = V_P - V_A$.

I used three basic tools of quantitative genetics: breeding schemes, artificial selection and inbreeding. By measuring the resemblance between relatives (as in a breeding scheme), one can determine the narrow-sense heritability and find indications for genetic correlations between traits. The response to artificial selection can help clarify the short-term potential for adaptive change and consequently the presence and distribution of additive variance for a trait, as well as the occurrence of genetic correlations between traits. By studying the effect of inbreeding in a population, the presence of directional dominance variance (deleterious recessive variation) can be determined and the occurrence of purging of this deleterious variation can be examined.
The role of selection in explaining additive variation

Life-history traits are expected to harbour little additive variance, because strong and constant directional selection will eliminate the additive variance for trait values that contribute less than maximally to fitness. However, additive variance is often found to be higher in life-history traits than in traits less closely related to fitness, such as morphological traits (e.g., Houle 1992; Kruuk et al. 2000; Merilä and Sheldon 2000; McCleery et al. 2004; Coltman et al. 2005). The reason for this pattern is believed to relate to the level of integration of a trait. A life-history trait is further down the causal pathway from genes to fitness (Price and Schluter 1991) and therefore integrates many more genes than morphological traits, which creates conditions for higher additive variance. Although additive variance is on average higher for life-history traits than for morphological traits, the amount relative to the total phenotypic variance (i.e., the narrow-sense heritability: $h^2 = V_A/V_P$) is usually lower (e.g., Houle 1992; Kruuk et al. 2000; Merilä and Sheldon 2000; McCleery et al. 2004; Coltman et al. 2005). This is caused by even larger differences in the non-additive variance (the residual variance), where both the environmental variance and the (dominance and epistatic) genetic variance can be larger. This higher residual variance of life-history traits is also believed to be caused by their higher integration level. Thus, large differences in residual variance dictate the patterns of narrow-sense heritability across life-history and morphological traits; structure – not selection – defines the genetic architecture across these trait classes. A subsequent question is whether the same patterns are visible within the class of life-history traits: are differences in heritability across exclusively life-history traits caused by differences in residual and/or in additive variance? (See p. 13, question 1 and Chapter 2.)

A related finding that is in disagreement with the prediction of low additive variance for a life-history trait is that the amount of additive variance differs greatly between life-history traits (e.g., Mousseau and Roff 1987; Kruuk et al. 2000; Merilä and Sheldon 2000; Stirling et al. 2002; McCleery et al. 2004) and can be very high (e.g., see Chapter 2). How can we explain that a trait under strong directional selection contains high additive variance? There are two processes through which natural selection can lead to high additive variance in life-history traits; the mutation-selection balance and balancing selection. Genetic variation is ultimately caused by mutation, but the strength and type of selection defines how long these alleles are present in a population. Most mutations lead to deleterious alleles, which are removed from the population within tens of generations (Lynch and Walsh 1998). The balance between input by mutation and removal by selection (the mutation-selection balance) leads to a constant source of genetic variation, however short individual alleles persist. Genetic variation can also be maintained by selection. This so-called balancing selection can occur when the fitness of different variants is frequency-dependent or
fluctuates temporally, through migration or when overdominant gene action plays a role. Most of these situations are not expected for a life-history trait, given the assumption of a direct relationship with fitness. However, genetic trade-offs can lead to more complex patterns of overall selection. If, for example, traits A and B have a negative genetic relationship and the relative importance of the traits for fitness is environmentally determined, then temporal variation in the environment can cause an overall maintenance of genetic variation for both traits. The nature of the genetic variation created by these two processes is fundamentally different: where a mutation-selection balance results in mostly deleterious variation, balancing selection maintains beneficial variation. It is therefore important to understand the relative contribution of both types of processes (Barton and Keightley 2002; Mitchell-Olids et al. 2007). Experimental work to unravel these contributions is scarce, however. (See p. 13, question 2 and Chapter 3.)

Directional dominance variance in diploids and haplodiploids

Another important research area in the evolutionary genetics of life-history traits concerns the effects of inbreeding. Mating with relatives often results in offspring with lower fitness, which is termed inbreeding depression. Inbreeding leads to increased homozygosity, which leads to (a) the expression of deleterious recessive alleles and (b) the decreased presence of heterozygote alleles with overdominant gene action (where heterozygotes have a higher trait value than homozygotes). A wealth of results shows that inbreeding depression is mainly caused by the expression of deleterious recessive alleles (Lynch and Walsh 1998; Roff 2002a; Charlesworth and Willis 2009).

Life-history traits are more affected by inbreeding than morphological traits (DeRose and Roff 1999). This larger effect is believed to be related to the strong directional selection working on life-history traits. All alleles for higher trait values quickly become fixed and additive and dominant alleles for lower trait values are eliminated. In contrast, recessive alleles for lower trait values can remain hidden in an outbred population in the heterozygote individuals. In traits that are only weakly related to fitness or under stabilizing selection, such as morphological traits, selection is less pronounced since alleles for slightly higher and slightly lower trait values will be selectively equivalent (Lynch and Walsh 1998). Thus, the amount of deleterious recessive variation — directional dominance variance — will be larger for life-history traits. As a consequence, the effect of inbreeding is larger in life-history traits.

Theory with regard to haplodiploid species (such as *T. urticae*) differs somewhat from the general theory on inbreeding and directional dominance (which is based on diploid species). Haplodiploids should harbour less directional dominance variance, because recessiveness does not play a role in the haploid gender. Alleles
of genes expressed in the males are thus constantly under purifying selection (Avery 1984; Werren 1993). This should result in less inbreeding depression in haplodiploids than in diploids (Hedrick and Parker 1997), which has been found in a meta-analysis carried out by Henter (2003). A complicating factor is that some genes are only expressed in females and can thus remain hidden from the purifying selection to which the haploid males are exposed (Crozier 1976; Werren 1993). If subsequently a life-history trait is controlled by many of these genes with female-limited expression, it may contain more directional dominance variance and incur more negative effects of inbreeding. Thus, in a haplodiploid species the effect of inbreeding on life-history traits may be related to gender. (See p. 14, question 3 and Chapter 4.)

The role of genetic trade-offs

Traits that are under directional selection should theoretically increase indefinitely. The evolutionary pathway of these traits is however constrained, both by phylogenetic and genetic constraints. One type of genetic constraints constitutes genetic trade-offs, whereby the advantage of a change in one trait is correlated to a disadvantage in other traits (Futuyma 2009). A central premise in life-history theory is that traits are limited by these genetic trade-offs (e.g., Stearns 1989; Roff 2002b; Roff and Fairbairn 2007): genetic trade-offs are major components in most optimality models and also in many genetic models (Futuyma 2009) and are for that reason alone important factors to study.

Trade-offs can be divided into two classes: those that act directly on life-history traits – for example between early- and late-life reproduction – and those that act indirectly via an underlying trait (Roff 2002b). The presence of a trade-off of the first type was investigated in this thesis with regard to explaining the heritable variation in oviposition rate (see subsection The role of selection in explaining additive variation). I also explored the genetic correlation of life-history traits with two behavioural traits that are central to the ecology of the two-spotted spider mite: web production and dispersal behaviour. Both traits can be vital for survival or reproduction. Here, the reasons are given per trait why we studied these potential trade-offs.

The primary defence against predators is formed by the production of vast amounts of silk, which results in a sticky and chaotic web. The webbing is predominantly produced by the adult females and necessitates the metabolism of large amounts of protein. An obvious potential genetic correlation involves a protein limitation: the high production rate of eggs of *T. urticae* also requires a high metabolism of protein, which may lead to an allocation trade-off between the two traits. (See p. 14, question 4 below and Chapter 3.)
With regard to dispersal, metapopulation theory suggests that in species that live in unstable environments (such as *T. urticae* does), dispersal traits may be strongly related to life-history evolution, especially reproductive traits (e.g., Olivieri et al. 1995; Ronce and Olivieri 1997). Empirical studies have demonstrated the presence of genetic correlations between dispersal and reproductive traits in various species (e.g., Roff and Fairbairn 2001; Fjerdingstad et al. 2007; Roff 2002b; Chapter 5). Previous work on dispersal in *T. urticae* suggests that dispersal also trades off with reproduction-related traits in this species (Yano and Takafuji 2002). However, alternative mechanisms (related to the experimental set-up employed) still needed to be excluded in order to make firmer statements on possible genetic trade-offs between dispersal and reproduction in this species. (See p. 15, question 5 and Chapter 5.)

**The two-spotted spider mite**

The two-spotted spider mite (*Tetranychus urticae* Koch) is an herbivore of about half a millimeter in length. It has four pairs of legs and a stylet to pierce plant cells. It has a haplodiploid sex-determination system, where the females have two sets of chromosomes (2n = 6) and two parents, whereas the males arise from unfertilised eggs and thus have one set of chromosomes and only a mother as parent. For females of *T. urticae* usually only the first mating results in fertilization (Helle 1967). With a nice Dutch summer temperature (25°C) a female can lay 12 eggs per day which reach adulthood within 12 days. There are seven developmental stages; the egg, the six-legged larva, two eight-legged nymphal phases, three intermediate moulting phases and the adults.

Adults (mostly the females) spin a silken web, which leads to a chaotic, sticky and dense shield against environmental fluctuations and predators (Saito 1985). The adults and their offspring live inside this shelter, which expands in size as more females become adult and start feeding and walking around. The silken web is the primary defensive system and has proven successful against many predatory mites (Sabelis 1985; Sabelis and Bakker 1992). *Tetranychus urticae* has one of the highest rates of web production of the Tetranychinae (Saito 1985) and can only be effectively preyed upon by specialist predators such as *Phytoseiulus persimilis*.

Dispersal is another central aspect of the spider mite’s ecology, because this species lives in highly unstable habitats. *Tetranychus urticae* has a very high rate of increase (a population can increase in size by 22-34% per day; Sabelis 1991), which leads to rapid overexploitation of its host plant. Predators such as *P. persimilis* can efficiently decimate local colonies of spider mites and survival is consequently dependent on dispersal to neighbouring plants (Ellner et al. 2001). Also, the mite is a major pest in agriculture and horticulture, where it faces frequent crop rotation and
pesticide treatment. For all these reasons, dispersal to new hosts is vital. Mostly adult females disperse, either swept away by air currents from the tips of the plant or by walking to neighbouring plants.

**Questions and summary of results**

1. *Can patterns in the genetic architecture across life-history traits be explained by structure and/or selection?*

   Selection decreases the additive variance for a trait and consequently also its heritability. Structure (i.e., the level of integration of a trait) increases additive variance but has an even stronger positive effect on residual variance and consequently works negatively on heritability. If structure is the main factor determining the genetic architecture of traits, then across traits heritability would be predominantly negatively related to residual variance. If selection is the main factor, heritability would be positively related to additive variance. In Chapter 2 the $h^2$ and the coefficients of additive ($CV_A$) and residual ($CV_R$) variance of life-history traits of a natural population of *T. urticae* were determined using a breeding scheme and plotted against each other in order to discern patterns in their relationship. A clearly positive relationship between $h^2$ and $CV_A$ was present, while no pattern could be discerned between $h^2$ and $CV_R$. Thus, within this narrow range of integration (i.e., only life-history traits), a signature of selection seemed present, while no effect of structure could be recognized.

2. *Which mechanism maintains high additive variance for oviposition rate?*

   Oviposition rate has high additive variance in two populations of *T. urticae* (see Chapter 2 and 3), despite the assumedly strong directional selection working on this trait. Can this heritable variation be explained by balancing selection and/or is it the result of the mutation-selection balance? If balancing selection is a dominant process, then the additive variance represents beneficial variation, while under a mutation-selection balance it represents deleterious variation. In Chapter 3, the relative importance of these two mechanisms was investigated for a laboratory population of *T. urticae*. A suite of results from three artificial selection experiments point towards the mutation-selection balance as the principal mechanism behind the high additive variance found for oviposition rate. Balancing selection was not found to be a dominant mechanism. Also, inherited variation caused by maternally inherited cytoplasmic bacteria did not play a role (see question 7). These results imply that the high additive variance for oviposition rate is predominantly deleterious variation and has no predictive power for the adaptive potential of the trait.
3. Is the effect of inbreeding related to gender?

In outbred populations of haplodiploid species the frequency of recessive deleterious alleles is expected to be higher for genes that are expressed exclusively in females than genes expressed in both females and males. Is then also the presence of directional dominance variance for life-history traits related to gender? And can the population be purged of this directional dominance variance through bouts of inbreeding? In Chapter 4 the effect of inbreeding on various life-history traits was examined through an inbreeding scheme. Inbreeding led to a reduction in oviposition rate and (male and female) maturity rate, while longevity and juvenile survival were not affected. The reduced male maturity rate cannot be explained by increased homozygosity in the male, since they are hemizygous. The effect can be explained by inbreeding effects in the mother, combined with maternal genetic control over the development rate of her offspring. This was corroborated by an absence of effect in inbred offspring of an outbred mother. Thus both traits that are affected by inbreeding seem to be affected through female aspects of the traits; oviposition rate is a female trait and maturity rate seems to be under maternal control. Purging through inbreeding occurred only with regard to oviposition rate. However, in this experimental set-up purging with regard to maturity rate cannot have occurred if it was a maternally controlled trait.

4. Are life-history traits genetically correlated with web production? And is there adaptive potential for web production?

Web production is an important aspect of the mite’s defense against predators but requires the input of large amounts of protein, which are also required by other fitness components such as oviposition rate. This may lead to a genetic trade-off that can hamper the evolution of web production and/or the linked fitness component. In Chapter 2, the presence of heritable variation for web production was explored in a natural population using a breeding design, together with a preliminary exploration of genetic correlations with life-history traits. In Chapter 3, a more thorough investigation of a potential genetic trade-off with oviposition rate was performed using artificial selection on oviposition rate. Heritability of web production was estimated to be reasonably high (Chapter 2), which implies that web production has the potential to adapt in this population. In contrast to the expectation of a negative genetic correlation with oviposition rate, indications for a positive relationship were found instead (Chapter 3). Thus, the evolution of oviposition rate does not seem to be hindered by a genetic trade-off with web production. The breeding scheme used to examine the presence of genetic trade-offs of web production with life-history traits (Chapter 2) did not deliver reliable data for examining genetic correlations: the statistical power of the correlations was too low to draw firm conclusions.
5. Are life-history traits genetically correlated with the propensity to disperse? And is there adaptive potential for this dispersal trait?

It is important to take dispersal into account when investigating the life-history evolution of *T. urticae*. Local populations of these spider mites often overexploit their host plants, which makes metapopulation dynamics essential for explaining the stability of its populations. Within this framework dispersal is thought to be important for life-history evolution, especially with regard to reproduction. Therefore, we investigated the adaptive potential of the propensity to disperse in a population of *T. urticae* as well as the presence of genetic trade-offs with life-history traits (Chapter 6). In an artificial selection experiment, no response to selection was found nor were correlated responses in life-history traits present. It is therefore not likely that this population of *T. urticae* harbours much heritable variation for propensity to disperse by ambulatory means. It is also not likely that the life-history traits examined for this population were hampered in their evolution by trade-offs with the propensity for ambulatory dispersal.

6. Do spider mites avoid inbreeding through disassortative mate choice?

The finding of inbreeding depression in *T. urticae* (see Chapter 4) led to the question whether this has triggered sexual selection for the avoidance of inbreeding. If mating with related partners leads to offspring with lower fitness, than selection may have caused spider mites to preferentially choose partners that are unrelated. In Chapter 5 we investigated the mate choice of adult *T. urticae*. An adult virgin female was placed with an unrelated male and a brother with whom she had shared her juvenile habitat. Overall, 65% of the matings took place between the unrelated individuals, which was found to be significantly different from random mating. This shows that *T. urticae* can distinguish brothers from unrelated conspecifics and can prefer to mate with an unrelated male, thereby preventing inbreeding.

7. Do intracellular bacteria cause inherited variation in their host’s life-history traits?

*Wolbachia* and *Cardinium* are cytoplasmic bacteria that are inherited maternally via the cytoplasm and can influence reproductive traits of their hosts (Vala et al. 2003). If not all females are infected, these bacteria might cause life-history traits in a population to harbour inherited variation that is not related to the genetics of the mite. The presence of such bacteria and their effect on oviposition rate were examined in Chapter 3, in order to investigate a possible role of these bacteria in explaining inherited variation for oviposition rate. In a laboratory population 56% of the females were infected with the cytoplasmic bacteria *Wolbachia*. However, no difference in
oviposition rate (or other examined life-history traits) was found between infected and uninfected females. *Wolbachia* and *Cardinium* thus did not cause inherited variation in these traits.

With the results summarized above I have tried to clarify the relationship between the genetic architecture of life-history traits and the selective forces working on these traits. How important is the role of natural selection in shaping the genetic variation for life-history traits? Which forms of selection take place over life-history components? How strong are the selective forces? Is the strength of selection related to gender? A discussion of this synthesis and some further literature research related to these questions are given in Chapter 7.

**References**


