Biogeography, population genetics and mating systems of natural Nasonia populations
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2009

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Grillenberger, B. K. (2009). Biogeography, population genetics and mating systems of natural Nasonia populations. s.n.

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Chapter 1

General Introduction

B. K. Grillenberger

Motivation of this thesis

Evolutionary biology aims at understanding the processes leading to the enormous phenotypic diversity we observe in nature and wants to reconstruct the evolutionary history of life on earth (Futuyma 1986b). This includes research on functioning of individual genes and gene-complexes as well as on environmental forces that lead to the adaptive evolution of these gene networks. Adaptation to a local environment can lead to ecological speciation, when the complexity and heterogeneity of the environment leads to reproductive isolation and ultimately the evolution of new species. One approach in evolutionary research is the development of models that describe the essence and most important features of the evolutionary process (Futuyma 1986a). In order to describe the enormous complexity of natural processes, two major types of models have been developed: (1) general models that are limited to a certain range of conditions and rely on many (sometimes optimistic) assumptions; (2) models that are mimicking a specific system and are only applicable to a narrow range of cases. The ultimate goal is to merge these two approaches and develop detailed models that are applicable in a wide range of scenarios. Especially for detailed models a lot of information about the biology of an organism is needed. A common approach to obtain this information is to focus on model organisms. Focusing on the biology of a single organism provides the advantage that many researchers can combine their results and build a more and more complete picture of a specific system. A model organism in evolutionary biology should be amenable to experimental analysis in fields such as ecology and genetics, be open to field studies, easy to be handled in the laboratory and have a short generation time.

The jewel wasp, *Nasonia*, has become a model organism in evolutionary biology, because of its ease of handling and rearing (Barrass 1976), and its
interesting biology. The genus *Nasonia* consists of three closely related species that are found in sympatry as well as in allopatry (see below). *Nasonia* has been used in a large variety of studies in behavioural (e.g. Barrass 1961; van den Assem & Visser 1976; King 1993; Beukeboom & van den Assem 2001; van den Assem & Beukeboom 2004; Leonard & Boake 2006; Lehmann & Heymann 2006; Shuker et al. 2006a), physiological (e.g. King 1962; Rivers & Denlinger 1995), developmental (e.g. Lynch et al. 2006; Olesnicky & Desplan 2007) and genetic (e.g. Gadau et al. 1999; Beukeboom & Werren 2000; Opijnen et al. 2005; Velthuis et al. 2005) research. A particular fruitful field of research has been sex allocation and sex ratio distorters (e.g. Werren 1980; 1983; 1988; Beukeboom & Werren 1992; Werren & Beukeboom 1993).

In the *Nasonia* system it is possible to compare and cross individuals between closely related species, as well as individuals of one species originating from natural populations that experience different environmental conditions (see below for details). Therefore the *Nasonia* system provides a unique opportunity to test the validity of models on speciation and adaptation.

Given the wide applicability of, and interest in, the *Nasonia* system, the genome of *N. vitripennis* and its sister species, *N. giraulti* and *N. longicornis*, has been sequenced. As a spin off of the sequenced genome many more studies on genetic and genomic level will follow, and application of molecular tools in parasitoid research will be intensified considerably. However, many ecological aspects and basic life history traits of *Nasonia* are still unknown. Factors and assumptions that are included in theoretical models have to be confirmed experimentally, to make these models realistic and to judge the validity of different models. So far, most experimental research on *Nasonia* has been conducted in the laboratory, but laboratory results can often be biased due to the use of a constant set of arbitrarily chosen laboratory conditions, that only resemble a fraction of the range of natural conditions. Hence, the wider applicability and relevance of the models to field populations remains largely to be determined.

To understand the life history of any organism it needs to be considered how events in different stages of the annual cycle interact and influence subsequent events at the level of the individual and, eventually, the population (Webster et al. 2002). To accomplish this, one needs to consider the complexity of an organism’s natural environment. One consequence of this complexity is that an
organism has to adapt to a large variety of local conditions, to attain optimal fitness. A precondition of the adaptation to local environments, and eventually speciation, is limited gene flow between different populations. If there is gene flow between local populations locally adapted allelic variants and co-adapted gene complexes might be under opposite selection in other habitats. This difference in selection pressure between local populations can eventually inhibit fixation of an adaptive allele in the population. To assess the probability of local adaptation and the potential for speciation, knowledge about migration between and admixture within populations is required.

In this thesis I provide information on the population genetic structure, phylogeography, reproductive strategies and dispersal abilities of field populations of *Nasonia*, in order to judge the validity of previous results on sex allocation behaviour derived from laboratory experiments under natural conditions. I also evaluate the precision of adaptation in sex and resource allocation of *Nasonia* in sympatric and allopatric populations.

**Local mate competition theory**

A very intensely studied field in evolutionary biology is sex allocation, and as part of that Local Mate Competition (LMC) theory. Due to its haplodiploid sex determination (see below for details) *Nasonia* became the preferred model species in this field. As a large part of the work presented in this thesis is motivated by LMC theory, I will introduce it here.

Fisher (1930) argued that in a large random mating population the only evolutionary stable strategy is an equal investment in the two sexes. The main reason for this is that, as soon as one of the sexes becomes more abundant in the population, it is advantageous to invest more in the rarer sex, because that sex will have a higher fitness on average. As this is true for either sex that is in the minority, the only stable outcome is an equal frequency of both sexes.

LMC theory (Hamilton 1967) gave an explanation for the observed biased sex ratios in nature, relaxing the Fisherian assumption of a large random mating population. This theory is the basis for a large amount of research into adaptive sex ratio adjustment (Taylor & Bulmer 1980; Werren 1983; Herre 1985; Orzack 1986; King & Skinner 1991; Hardy 1994; Godfray & Werren 1996; Antolin 1999; Courteau & Lessard 2000; West *et al.* 2000; Shuker *et al.* 2004b; 2005). The basic assumption of LMC is, that if a female has control over the sex ratio
of her offspring, she can increase her fitness by reducing the competition between her sons. This is an evolutionary stable strategy if females are the only dispersing sex and if mating only takes place at the natal patch (Hamilton 1967). In such a system competition takes place between all local males to mate with females that are available at the patch. If the population is founded by a single female only, all males are brothers and it is beneficial for the founding female to shift the offspring sex ratio strongly towards daughters to reduce local mate competition between her sons, and in order to maximize the offspring yield in the next generation (her grandchildren). With increasing foundress number, competition between unrelated males increases and therefore selection favours females that produce more males to increase the chance that their sons mate with daughters of other females and hence contribute to an increase of the mother’s genes in the next generation. This leads to a less female-biased sex ratio. The resulting prediction is that the offspring sex ratio in a patch is a function of the number of females ovipositing in that patch, starting at very low sex ratios (proportion of males) with few foundresses and approaching a balanced sex ratio at very high foundress numbers (Hamilton 1967) (see Figure 1.1).

A central idea of LMC theory is that mating exclusively takes place within the patch, and therefore the population is highly structured, and inbreeding is high. In the case of parasitoids the structuring is thought to be due to the patchy distribution of hosts. With limited dispersal the relatedness among the founding females would increase, and therefore the population inbreeding would be high, leading to an increase of relatedness among competing sons of multiple foundresses in a patch. The consequence would be an increase of inclusive fitness benefits for the individual foundresses via the offspring of their related co-foundresses, which in turn leads to a lower optimal sex ratio (less males). Inbreeding has been considered in several extensions of the basic Hamilton model (Herre 1985; Frank 1985b). Nunney and Luck (1988) modelled the combined effects of male dispersal, inbreeding and asynchronous parasitism on sex allocation, whilst Courteau and Lessard (2000) developed several different scenarios of dispersal, i.e. before or after mating and dispersal probability for haploid, diploid and haplodiploid organisms.
While most authors focused on the actual number of foundresses parasitizing a patch, Werren (1980) considered the relative clutch size of a second parasitizing female compared to the first female to be the crucial factor in sex ratio adaptation. Shuker et al. (2005) recently extended Nunney and Luck’s (1988) model of asynchronous parasitism by considering two foundresses parasitizing hosts on a patch sequentially but allowing females to use either the same or different hosts. In species such as the parasitic wasp *Nasonia vitripennis* asynchronous parasitism on a single host is thought to have little effect on the timing of emergence, as *N. vitripennis* larvae of later deposited eggs speed up their development to achieve a synchronous emergence of all individuals from a host (Werren 1980). In contrast, asynchronous parasitism of several hosts in a patch leads to asynchronous emergence of the offspring, as each host has a different emergence time. As such, males of an early foundress have a chance to mate with females of a later foundress, whose sons do not have access to the daughters of the early foundress. Such asymmetric LMC leads to a shift of the optimal sex ratio towards more males for the second foundress, compared to previous models (Shuker et al. 2005). To what extent asynchronous parasitism is taking place in nature is largely unexplored.

LMC theory has been tested on a number of parasitic wasp species. Among others *Pachycrepoides vindemiae* (Nadel & Luck 1992), *Muscidifurax raptor* (King & Seidl 1993), and *Spalangia endius* (King 2002) show a clearly female...
biased sex ratio, but follow only roughly LMC theory. Since in all three species males can fly and disperse facultatively and therefore do not obey to the primary criteria of LMC, they may experience only partial LMC. Studies on these species show that next to LMC, host quality appears to play an important role in sex allocation (Nadel & Luck 1992; King & Seidl 1993; King 2002). Several field studies on fig wasps support the idea of LMC, but there is a lot of variation and many more factors than just the number of foundresses seem to play a role (Herre 1985; Herre 1987; West & Herre 1998; Moore et al. 2002; Greeff 2002; Pereira & do Prado 2005). As a general trend Herre (1987) found that those species that regularly encounter high foundress numbers in a patch produce sex ratios that are closer to the predictions of LMC theory than species that usually parasitize with a single foundress.

*N. vitripennis* has been widely used for laboratory experiments regarding sex ratio adjustment (Werren 1980; 1983; 1984; Drapeau & Werren 1999; Shuker & West 2004; 2004a; Shuker et al. 2004b; 2005). Laboratory experiments and two field studies (Werren 1980; Molbo & Parker 1996) have shown that *N. vitripennis* modulates the sex ratio of its offspring broadly consistent with LMC theory. However, to what extent the experimental laboratory conditions resemble the field situation is little known. One important condition for LMC to occur is that *Nasonia* regularly encounters competitors while parasitizing hosts. The only information on superparasitism rates comes from a small field study in Sweden using allozyme markers (Molbo & Parker 1996). These data are the first in documenting foundress numbers in a natural situation. However, allozymes have limited resolution and may underestimate the actual number of foundresses. Hence there is a need for more field data to evaluate the complementarities of population structure and dynamics of LMC theory. Since the parasitoid wasp *Nasonia* has already proven to be an ideal model to test LMC theory, it is the species of choice in this study. In the following section I will introduce the *Nasonia* system in more detail.

**The Nasonia model system**

*Nasonia* is an approximately 3 mm large pupal parasitoid of cyclorrhaphous flies, which are mostly found in bird nests and on carcasses (Whiting 1967). Up to now, three species are known in the genus *Nasonia*: *N. vitripennis* seems to be cosmopolitan (Whiting 1967, and see: http://www.nhm.ac.uk/research-
curation/projects/chalcidoids/), *N. giraulti* is found in the north-eastern and *N. longicornis* in the north-western part of North America (Darling & Werren 1990). Due to the patchy distribution of its host, populations of *Nasonia* are thought to be extremely substructured (Darling & Werren 1990; Molbo & Parker 1996), with the consequences for sex ratio adaptation discussed above. The major morphological difference between the species is the male wing length: *N. vitripennis* has short wings, *N. giraulti* has long wings, and *N. longicornis* intermediate wings (Darling & Werren 1990).

The divergence of the three species is thought to be a relatively recent event. *N. giraulti* and *N. longicornis* are estimated to have separated from *N. vitripennis* around 1 Myr ago. The separation of *N. longicornis* and *N. giraulti* is placed around 0.25 Myr ago (Campbell et al. 1993). The species are reproductively isolated due to infection with incompatible *Wolbachia* strains, which causes chromosome destruction in interspecific crosses (Breeuwer & Werren 1990). However, cured strains produce viable and fertile hybrid offspring (Breeuwer & Werren 1995). Behavioural studies have shown that there are clear differences in courtship behaviour between the three species leading to prezygotic isolation (van den Assem & Werren 1994; Beukeboom & van den Assem 2001).

It is assumed that the cytoplasmic incompatibility caused by *Wolbachia* infection played a major role in the initiation of the speciation process (Bordenstein & Werren 1998; Bordenstein et al. 2001). However, it remains to be established whether *Wolbachia* was the primary cause or has played a role in the maintenance of genetic separation leading to speciation. It is also not known whether the speciation of *N. longicornis* and *N. giraulti* took place in allopatry or in sympatry with *N. vitripennis* in North America (see below).

The life cycle of *Nasonia* is similar in all three species (Figure 1.2): An adult female (a foundress) searches for host pupae and drills a hole in the puparium with her ovipositor. She stings the host and injects venom into the host pupa, which will eventually kill the host. She lays about 30 eggs, depending on host size and the presence of other female’s eggs, directly onto the fly pupa. The eggs hatch inside the host puparium and upon emergence the larvae feed on the body tissue of the host. After two weeks of development at 25ºC, the adult wasps are ready to emerge. Males reach adolescence earlier and emerge shortly before females. They bite their way through the puparial shell and wait at the
exit hole for the females to emerge. Once the females have crawled out of the puparium, the males start courting them. As a consequence, the amount of inbreeding depends on the number, relatedness and produced sex ratios of foundresses that have laid their eggs in a particular host. After mating the females usually disperse and search for a new host patch. In *N. vitripennis*, males have reduced wings and are thought to stay at their place of birth to wait for other females to emerge, until they die (Whiting 1967). It has been found that *N. longicornis* males stay at the mating site as well, while *N. giraulti* males have a tendency to disperse after mating (Leonard & Boake 2006). However, how far *N. giraulti* males disperse, and whether there is a chance for them to find a mate, has not been tested yet.

The mating behaviour after emergence is generally similar for all three species, but especially *N. giraulti* and to some degree *N. longicornis* females mate within the host before emerging (Drapeau & Werren 1999). Within Host Mating (WHM) is rare in parasitoids and the occurrence of this behaviour in natural populations of *Nasonia* would have a large influence on the population structure. In the case of a single parasitized host, the offspring will perform exclusively sib-mating. If most hosts are single parasitized, the degree of inbreeding in the population would therefore be increased if WHM occurs regularly. It has been suggested that WHM is an adaptive behaviour of *N. giraulti* to avoid hybrid mating with *N. vitripennis* (Leonard & Boake 2006). This hypothesis still remains to be tested.
Figure 1.2: Schematic life cycle of *Nasonia vitripennis*

*Nasonia* has haplodiploid sex determination. Fertilized eggs develop into females and unfertilized eggs into males. After mating sperm is stored in a spermatheca, and due to its anatomy, females are able to facultatively fertilize an egg. This enables females to control the sex ratio of their offspring. The biology of *Nasonia* seems to fit most assumptions of Hamilton’s LMC theory and the species has been widely used in LMC research (see above). However, the details of the cues that are influencing sex allocation decisions and the underlying mechanisms are not well understood yet.

As mentioned above, the major morphological difference between the three species in the genus *Nasonia* is the wing size of the males (Darling & Werren 1990). *N. vitripennis* has short wings, *N. giraulti* has long wings that cover the abdomen and *N. longicornis* has intermediate sized wings. The males of *N. vitripennis* are incapable of flight, while those of the other two species can fly, and might therefore disperse (King & Skinner 1991; Leonard & Boake 2006; Lehmann & Heymann 2006). However, there is a report of *N. vitripennis* males
and females mating six meter away from their natal host (Grant pers. comm. in Orzack 1986). The dispersal abilities of *Nasonia* have not been examined in detail yet, but one can imagine that such a small insect might have problems to cover long distances by its own power. If dispersal abilities are limited, the strong population substructuring would lead to very low gene flow between subpopulations and therefore inbreeding should be very high within the subpopulations. If *Nasonia* can disperse by wind drift, as reported for fig wasps (Harrison 2003), migration between the subpopulations would be higher and the level of inbreeding within the subpopulations be reduced. For *N. vitripennis* a high level of inbreeding has already been shown in one natural population using allozyme variation (Molbo & Parker 1996), but it is likely that different habitats induce a different dispersal behaviour in *Nasonia*, affecting the population structure. In addition, allozymes may not have enough resolving power to produce reliable estimates of inbreeding in populations with such a complex life history as *Nasonia*. For the other two species, *N. longicornis* and *N. giraulti*, information on inbreeding and dispersal are totally lacking.

As many insect species (Danks 2007), *Nasonia* larvae have the ability to survive unfavourable environmental conditions in a dormant state, called diapause (Whiting 1967). In most species it is the larva itself that induces and enters diapause upon experiencing unfavourable conditions. In *Nasonia*, however, diapause is induced in the mother but developmental arrest takes place in the fourth larval instar of her offspring. Several cues have been found to trigger the female to produce eggs that will develop into diapausing larvae: low temperature, long dark periods, food shortage and older age (Saunders 1962; 1965a; 1966a; 1966b). The diapausing larvae are able to survive up to two years and then continue normal development into an adult individual. In the laboratory diapause can be broken by exposure to low temperatures (~4ºC) for three months, and subsequent culturing at normal temperature (20-25ºC) (Whiting 1967).

The differences in life history, mating system and dispersal capabilities between the *Nasonia* species, and the various environments in which *N. vitripennis* can be found (sympatric with its sister species in North America, and allopatric in Europe), provide unique opportunities to study the precision of adaptation. In this thesis the main focus will be on the precision of two adaptive traits: sex allocation and diapause.
Population history of Nasonia

The origin and fixation of locally adapted traits in a population usually takes a long time. To estimate the probability of local adaptation it is necessary to obtain information on the population history of a species. In principle two histories of the colonization of the North American continent by Nasonia are conceivable (see Figure 1.3): (1) The ancestor of all three Nasonia species invaded the New World and the speciation into the three species N. vitripennis, N. longicornis and N. giraulti took place sympatrically. N. vitripennis then extended its range from North America to the rest of the globe. If the species complex evolved in this way, high genetic variation in North America and a reduced variation in the rest of the world would be expected, due to a possible population bottleneck during colonization. (2) The ancestor of N. giraulti and N. longicornis came to North America and the speciation of these two sister species took place in isolation of N. vitripennis, which evolved outside the New World. In more recent times N. vitripennis was introduced to North America. In this case, the genetic diversity of the North American N. vitripennis population would be reduced compared to the European population, due to a possible bottleneck during the colonization event. The time that N. vitripennis lives in sympatry with one of its sister species has implications for the time during which behavioural adaptations may have evolved in response to interspecific competition. The population history of N. vitripennis in North America has not been investigated yet.
The goal of this thesis is to evaluate the underlying assumptions of models describing adaptive behaviour (sex allocation and diapause). I aim to acquire information on the precision of adaptation in a natural environment and gain a better understanding of the multiple selective forces that shape life history traits. The results of this study will help to place the many results of theoretical and laboratory studies in an ecological context and can help to identify remaining questions about parasitoid life history evolution.

**Methodological approach**

When working with small insects, like *Nasonia*, direct observational methods are limited to the laboratory. For field observations the many techniques that are commonly used with larger species (e.g. radio tracking, colour banding, mark-release-recapture over longer time spans) cannot be applied. An alternative to answer many field biological questions is an indirect approach using genetic markers. The choice of the marker is dependent on the application, as different markers have different strengths and weaknesses. In the following I will provide more information and justification for the two types of markers that are used in this thesis: nuclear microsatellites and mitochondrial DNA sequences.

**Microsatellites**

Microsatellites, or simple sequence repeats (SSRs), are non-coding repetitive DNA sequences consisting of short (1 to 6 bp) repeats which occur in nuclear
and organelle DNA and are widespread across all eukaryotic genomes. Due to their high mutation rate and, in general, selective neutrality these markers have been broadly used in population genetic studies and for paternity analysis. The allelic differences are caused by variation in the number of repeats in the repetitive sequence, which is scorable as the difference in amplified fragment lengths from primer sets flanking the repetitive sequence. These repetitive sequences can be scanned for using a molecular probe, and therefore it is possible to develop primer for microsatellites even without having the genome sequenced. This makes microsatellites a very efficient marker for various applications, especially in non-model organisms.

The statistical tools that can be used to estimate population genetic parameters from microsatellite data are based on different mutation models (see Box 1.1). The most commonly used tool is Wright’s \( F \)-statistic (Wright 1931), which assumes an infinite number of possible alleles (infinite alleles model, IAM). The so called \( R \)-statistic (Slatkin 1995) is the counterpart to the \( F \)-statistic and is based on the stepwise mutation model (SMM). However, from discussions in recent literature it appears that most microsatellites follow a mixture of IAM and SMM (two phase model) and therefore neither the \( R \) nor the \( F \)-statistic would capture the truth. Therefore it is advisable to compare \( R \)- and \( F \)-statistical parameters and then draw conclusions (Oliveira et al. 2006).

A possible problem that can arise when using microsatellites is their enormous variability. Assuming a SMM and a limited number of possible alleles, the high mutation rate leads to a high chance of homoplasy over longer time spans. This results, over time, in an increasing unreliability of diversity and differentiation estimates (Nauta & Weissing 1996). Another problem of the high variability of the marker is the comparability of results between different studies. However, this problem seems to be largely solved with the invention of variation corrected differentiation estimates (e.g. \( G'_{ST} \)) (Hedrick 2005).
Box 1.1: Overview of the proposed mutation models in microsatellites following Oliveira et al. (2006):

1. Infinite alleles model (IAM):
   Every mutation alters the length of the microsatellite randomly, irrespective of the magnitude of the change. Therefore repeat units of length 5 and 15 are as closely related as lengths 5 and 6.

2. Step wise mutation model (SMM):
   During mutation, a microsatellite only loses or gains a single repeat unit. Therefore the genetic distance between two alleles is roughly proportional to the length difference between them.

3. Two phase model (TPM):
   A mixture of the IAM and SMM. The length of a microsatellite changes gradually (SMM), but occasionally larger leaps are possible.

4. K-alleles model (KAM):
   The above mentioned models assume an infinite number of possible microsatellite lengths (alleles). In contrast, the KAM assumes a limited number of alleles (k) possible for a microsatellite locus.

On the other hand, the high mutation rate ($10^{-2} – 10^{-6}$) in microsatellites rapidly creates differences between isolated populations, and when applied in the appropriate time scale, the resolution obtained by these markers can hardly be met with another marker type at the time this project was started. Another property is that every microsatellite locus appears to have its own mutation rate and pattern, depending on the number of repeats and its location in the genome. This leads to variation in the resolution between microsatellite loci, and makes it necessary to test the markers for their suitability to answer a specific question. However, this variation in resolution allows to find microsatellite loci with enough variation to differentiate between close relatives, but also to find loci that are conserved enough to address questions on inter-species level.

Considering the above mentioned properties, microsatellites are the markers of choice for the analysis of population structure and relatedness on a regional scale. With the recent advances in high throughput sequencing more effective methods are becoming available, but at the time this research was started, they were not an option.
Mitochondrial DNA sequences

Mitochondrial DNA (mtDNA) is the circular genome of mitochondria of eukaryotes. Every mitochondrion has one copy of it, and as there are many mitochondria in one cell, there are multiple identical copies of mtDNA per cell. There are 13 genes encoded by the mtDNA that are in close functional connection to nuclear encoded genes. Consequently, there can be a close association between certain mitochondrial types and nuclear genes.

Mitochondria are exclusively inherited via the maternal lineage, as they are transferred through the cytoplasm of the oocyte. There can be interactions between mitochondria and other cytoplasmic symbionts, leading to indirect selection on certain mtDNA haplotypes.

One problem of inferences from mtDNA can be the exclusive maternal transmission. In the case of differences in migration behaviour between males and females, or a different population history of the sexes, mtDNA can only reflect the female side of the story. Another pitfall in reconstructing population histories by mitochondrial information only, is its possible linkage to inherited symbionts, leading to a bias in the information that can be obtained (Hurst & Jiggins 2005). In *Nasonia* the symbiont is *Wolbachia*, which is exclusively maternally inherited as well (Werren 1997).

With $10^8 - 10^{10}$ substitutions per site and generation, depending on the gene, mtDNA is evolving fast and linear within a time frame of up to 150 Myr (Mueller 2006). It can easily be amplified (no cloning necessary) and, due to highly conserved regions, there are universal primers available, that facilitate the use in non model organisms.

These properties qualify mtDNA sequences to be the marker of choice for intraspecific inferences on a large scale, and for questions regarding the history of closely related species. I will use the information obtained from mtDNA in combination with microsatellites to combine the advantages of both markers, and to circumvent some of the problems when relying on only one type of marker.

Outline of this thesis

As outlined above, in spite of all advances in theoretical and laboratory research there is a profound lack of information on the natural ecology and behaviour of
parasitoids like *Nasonia*. The aim of this thesis is to fill some gaps between theoretical models and the natural situation in *Nasonia* research. In the following I will give an overview of the topics dealt with in the individual chapters of this thesis.

As described above *Nasonia* has been found to follow quite closely the predictions of local mate competition (LMC) theory in the laboratory. However, LMC theory is based on many assumptions about the population genetic structure, and it is unknown to what extent they are validated in nature. In **Chapter 2** (Genetic structure of natural *Nasonia vitripennis* populations: validating assumptions of sex ratio theory) I will evaluate the assumptions made in sex ratio theory on data from two European *N. vitripennis* field populations. In particular I will address the population structure, foundress number per patch, parasitization sequence and clutch sizes. As a follow-up, I will investigate in **Chapter 3** (Facultative sex ratio adjustment in natural populations of wasps: cues of local mate competition and the precision of adaptation) to what extent the predictions made by LMC theory are matching the sex ratios observed in the European populations and where the strengths and weaknesses of present models are.

Whereas in Europe *N. vitripennis* has no closely related competitors, the situation in North America is more complex. Given that *N. vitripennis* and *N. giraulti* are living in close sympatry in parts of North America, the question arises whether adaptations have evolved to avoid hybridization between the species. While it has been found that there are clear differences in courtship behaviour, it is still unknown whether there are adaptations with respect to LMC. In **Chapter 4** (Reproductive strategies under multiparasitism in natural populations of the parasitoid wasp *Nasonia* (Hymenoptera)) I will investigate the reproductive strategies of *Nasonia* in a two species situation, regarding the sex ratio adjustment as well as diapause production, focusing on how well *N. vitripennis* is adapted to the competition with its close relative *N. giraulti*.

In order to evaluate how far adaptation of *N. vitripennis* to the competitive situation in North America might have progressed, information is required on the population history of *Nasonia* in North America. So far, it has been assumed that the cosmopolitan species *N. vitripennis* has its origin in North America, as that seems to be the hot spot of diversity within the genus *Nasonia*. In **Chapter 5** (Population history of *Nasonia vitripennis* (Hymenoptera) in North America)
I test the hypothesis that *N. vitripennis* originates from North America, or from outside the New World. Using a combination of mtDNA, nuclear microsatellites and *Wolbachia* sequences, I am comparing the genetic variability among North American and European samples.

A prerequisite of local adaptation is that there is only limited gene flow between areas with and without selection pressure on the adaptive trait. *N. vitripennis* might have evolved adaptations to the presence of its sister species *N. girauldi* and *N. longicornis* in North America. However, as there are large areas in North America where *N. vitripennis* occurs allopatrically, without selection for competition with a close relative, the question arises whether local adaptation in the sympatric areas is possible. In this context I investigate in Chapter 6 (Female dispersal and isolation-by-distance of *Nasonia vitripennis* (Walker) in a local mate competition context) the dispersal capabilities of *N. vitripennis* on a local scale with a mark-release-recapture experiment as well as on larger scale with molecular markers.

In the final Chapter 7 I will merge the results of the previous chapters and sketch the current knowledge of the population structure and history of *N. vitripennis* in Europe and North America.

**Acknowledgements**

I would like to thank Leo Beukeboom, Louis van de Zande and Jürgen Gadau for all the helpful comments that considerably improved this chapter.