JYVÄSKYLÄ STUDIES IN BIOLOGICAL AND ENVIRONMENTAL SCIENCE

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The Evolution and Maintenance of Reproductive Strategies in Bag Worm Moths (Lepidoptera: Psychidae)

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Esitetään Jyväskylän yliopiston matemaattis-luonnontieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ambiotica-rakennuksen salissa (YAA303)
maaliskuun 13. päivänä 2004 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Mathematics and Science of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

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Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-9672-7
ISBN 978-951-39-9672-7 (PDF)
ISSN 1456-9701

Jyväskylän yliopisto, 2023

ISBN 951-39-1744-4
ISSN 1456-9701

Jyväskylä University Printing House, Jyväskylä

ABSTRACT

Kumpulainen, Tomi
The evolution and maintenance of reproductive strategies in bag worm moths (Lepidoptera: Psychidae)
Jyväskylä: University of Jyväskylä, 2004, 42 p.
(Jyväskylä Studies in Biological and Environmental Science,
ISSN 1456-9701; 132)
ISBN 951-39-1744-4
Yhteenveto: Lisääntymisstrategioiden evoluutio ja säilyminen pussikehrääjillä (Lepidoptera: Psychidae)
Diss.

Maintenance of sex is one of the central questions of evolutionary ecology. This thesis examines the evolution and maintenance of sexual and asexual reproduction in *Dahlica* and *Siederia* bag worm moths (Lepidoptera: Psychidae). Bag worm moths are taxonomically complex and ecologically poorly known species. *Dahlica* (Sexual and asexual) and *Siederia* (strictly sexual) are closely related, morphologically and ecologically very similar, and co-occur in same habitats. These species are particularly interesting due to their peculiar life cycle (long larval phase, short adulthood), female winglessness, and the occurrence of parthenogenesis, which is relatively rare among other Lepidoptera. A phylogenetic study with mtDNA markers questioned the current taxonomic classification of genera *Dahlica* and *Siederia* and supported multiple origins of parthenogenesis within this group of species. While co-occurrence of several bag worm moth species in the same habitats was common, the proportions of sexual and asexual reproduction varied from 0 to 100 % between populations. Parasitoid prevalence also showed considerable variation among different moth populations. The prevalence of sexual reproduction correlated positively with parasitoid prevalence across the study populations, suggesting that parasites select for sexual reproduction. An additional cost of sexual reproduction was indicated in two sexual bag worm moth species by a decreased number of fertilised eggs if males had previously copulated. In small, often female biased, populations this decrease may reduce the survival of sexual moths compared to co-existing asexual competitors. In a genetic analysis, populations of asexual *Dahlica fennicella* had lower genotype diversity but higher heterozygosity levels than their most common sexual competitors *Siederia rupicolella* and *Dahlica charlottae.* Clonal diversity was surprisingly high among asexual populations, probably explaining why asexuality has not led to an evolutionary dead end in *D. fennicella.* Among sexual *D. charlottae* populations, parasitoid prevalence was higher in the most inbred or genetically less varied populations supporting the importance of high genetic diversity of the host. Parasitoid prevalence in bag worm moth populations is also related to the abundance of ants in their habitats. In ant behaviour experiments, I found that wood ants (*Formica rufa* group) aggressively attack parasitoids while bag worm moth larvae remain unharmed. Bag worm moths were abundant in habitats where wood ants were abundant and, within habitats, in trees where wood ants were most numerous. These results suggest that wood ants indirectly protect bag worm moth larvae. While apparently ideal prey for wood ants, the larvae escape ant predation through low detectability while their most harmful enemies are readily attacked by the ants. Bag worm moths were not previously known to be ant-associated in any way. These studies offer new empirical evidence of the diversity of factors affecting the occurrence and competitive abilities of closely related sexually and asexually reproducing species.

Key words: Asexual; cost of sex; electrophoresis; evolution of sex; fertilisation; parasites; parthenogenesis; Psychidae; red queen hypothesis; reproductive strategies; sexual; *Wolbachia.*

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LIST OF ORIGINAL PUBLICATIONS

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This thesis is based on the following papers, which will be referred to in the text by Roman numerals I - V. I am the main writer in the papers I, II, III and V and I have performed large part of the work in each paper.

I Kumpulainen, T., Grapputo, A. & Mappes, J. Phylogeny and the evolution of parthenogenesis in Finnish bag worm moth species (Lepidoptera: Psychidae) based on mtDNA-markers. Submitted.

II Kumpulainen, T., Grapputo, A. & Mappes, J. Parasites and sexual reproduction in psychid moths. Evolution, in press.

III Kumpulainen, T., Mappes, J. & Grapputo, A. Multiple mating of males decreases fitness of females: Additional cost of sex in psychid moths. Manuscript.

IV Grapputo, A., Kumpulainen, T., Mappes, J. & Parri, S. Genetic diversity and parasitoid prevalence in populations of asexual and sexual bag worm moths (Lepidoptera: Psychidae). Submitted.

V Kumpulainen, T., Mappes, J. & Grapputo, A. Ants have positive effects on the abundance of untended bag worm moths (Lepidoptera: Psychidae). Manuscript

1 INTRODUCTION

1.1 Sexual and asexual reproduction

The occurrence and maintenance of sexual reproduction (sex) is one of the most intriguing questions in evolutionary biology. It has puzzled the researchers, students and a diversity of other people during the history of evolutionary theory. It is not surprising, however, that we still do not know what causes sex to persist, in particular if many of us consider sex as a synonym for reproduction or a subset of reproduction, as Ghiselin (1974) pointed out. Literally, sex means a form of reproduction with changes in the state of a cell or an individual, while reproduction is a change in the number of individuals (Bell 1982).

Asexuality is a form of reproduction where new offspring are produced by an individual alone without combining the genome of two separate individuals. Sexual reproduction, in contrast, produces offspring with a genome that is not a product of a single individual, but a new combination originating from two different parent individuals. Sexuality (or amphimixis) is characterised by meiotic division to produce haploid gametes, by an exchange of genetic material between homologous chromosomes in crossing over (genetic recombination) and by the fusion of two meiotically produced haploid gametes that originate from different parent individuals (Bell 1982). Thus sexuality is characterised by the exchange and re-organising of genetic material. In asexuality, meiosis, crossing over and recombination are often lacking and thus the asexually produced offspring are created by mitotic divisions that produce cells identical to those of the parent (see Maynard Smith 1978, Bell 1982).

Asexual reproduction is the original type of reproduction and sexuality is a mode of reproduction derived from asexuality. Later on, asexuality re-emerged in many forms such as parthenogenesis, i.e. reproduction without fertilization, in many higher taxa that had reproduced sexually. Sexuality most likely evolved from asexuality not for reproduction per se (see also Bell 1982) but to produce genetically varied offspring.

1.1.1 Occurrence of sexual reproduction

Among single-celled organisms such as bacteria and yeasts, asexual reproduction is the predominant mode of reproduction. Among more complex organisms, however, sexuality is more common and among higher plants and animals it is the most common type of reproduction. However, there is no common consensus of whether or not the sexuality of eucaryots and procaryots is of common origin. The most primitive sexual organisms such as algae and protists are usually isogamous, meaning that there is no differentiation between female and male gametes (see e.g. Maynard Smith 1978, 1998). The evolution of sexually reproducing organisms from asexuals with isogamous (equally sized and shaped) gametes has however proceeded to anisogamy where there are two types of gametes. In the higher animals and plants, all sexually reproducing (amphimictic) species have two types of gametes: larger, often immotile, egg cells and smaller, motile, sperm cells (Maynard Smith 1978, Bell 1982). The origin of the two different sexes: males and females, is based on the evolution of anisogamy from isogamy (see Maynard Smith 1978, Bell 1982).

Many species, e.g. numerous higher plants, are still capable of both asexual and sexual reproduction, at least to some extent, and many animals, such as cladocerans and aphids, regularly cycle from asexual to sexual reproduction and vice versa (see Maynard Smith 1978, Bell 1982). Certain forms of asexuality such as vegetative growth and vegetative reproduction of plants and colony forming animals may cause difficulties even for detecting the reproductive events. Among some species, strictly asexual and sexual reproduction may be difficult to separate, because in simultaneous hermaphrodites for example, meiosis and fusion of gametes actually take place, even if this takes place within one individual. Perhaps the most well-known examples of this include hermaphroditism in snails (see Bell 1982 and references therein)

Most insect species reproduce sexually. Although many insect species are able to reproduce parthenogenetically either as their primary or secondary reproductive strategy, only a small proportion of insects are strictly parthenogenetic. Differences between males and females are among the most conspicuous consequences of sexual reproduction and these features should be absent in taxa derived from asexual ancestors only. Thus, it is widely accepted that asexual reproduction among insects is derived from sexually reproducing ancestors (see Suomalainen 1962, Maynard Smith 1978, Bell 1982). However, among certain taxa such as social hymenopterans (e.g. Formicidae) and cyclically parthenogenetic aphids (Homoptera: Aphidodea), asexual reproduction through parthenogenesis is very common; while in other taxa, such as Lepidoptera, asexual reproduction is very rare and mostly takes place as secondary reproductive strategy (Suomalainen 1962, Bell 1982).

According to Maynard Smith (1998) the relative rarity of parthenogens in higher animals (including insects) and plants is because several genetic changes are needed for successful parthenogenetic varieties to arise. To be successful, the varieties are most likely apomictic because these will be able to retain the heterozygosity level of the parent (see 1.1.2 Parthenogenetic reproduction, paragraph 3). In the long run, however, also such varieties are likely to go extinct, because of mutation accumulation and poorer evolutionary abilities (see 3.1.2 Maintenance of sexual reproduction).

1.1.2 Parthenogenetic reproduction

Parthenogenesis does not necessarily mean pure asexual reproduction, which would lack meiosis and subsequent fusion of the gametes. Literally, it means "virgin reproduction", which happens without male contribution to the reproductive output (Bell 1982). Generally and in this context, it is used to cover all reproduction that does not require fertilisation by gametes produced by other (male) individuals (see Bell 1982, Maynard Smith 1998).

Parthenogenesis occurs either as obligatory or as facultative parthenogenesis (tychoparthenogenesis or tychothelytoky) (Suomalainen 1962, Maynard Smith 1978, Bell 1982). Obligatory parthenogenesis means that once parthenogenesis has evolved within a species, sexual reproduction becomes impossible. The species is obligated to reproduce by parthenogenesis. Most non-cyclically parthenogenetic species are obligatorily (and often apomictically) parthenogenetic (see Suomalainen 1962, Maynard Smith 1978, Bell 1982). However, in the absence of fertilisation many sexually reproducing insects are able to lay a few eggs, some of which develop into adults (Suomalainen 1962, Bell 1982). This facultative parthenogenesis may function as a secondary reproductive strategy due to its automictically parthenogenetic mechanism (see below) and low number of eggs that develop without fertilization. In a few species, such as facultatively parthenogenetic stick insects (Orthoptera: Phasmidae), the proportion of developing eggs may be very high (up to 99 %) (Maynard Smith 1978, Mantovani et al. 1996).

Special cases of parthenogenesis include gynogenesis (or pseudogamy) and hybridogenesis. In hybridogenetically reproducing species, sperm shells are needed to activate the development of the egg shell but the genome of the sperm shell is expressed in the embryo (Hubbs & Hubbs 1932, Maynard Smith 1978, Bell 1982). Females do not undergo meiosis, but mate with males of other species. The haploid genome of the sperm is first included in the diploid ova but is later excluded from the germ line of their daughters (Bell 1982). Hybridogenesis has been reported from *Poecilia* and *Poeciliops*is fish (Maynard Smith 1978 1998, Bell 1982) and from the frog *Rana esculenta* (Bell 1982, Som et al. 2000). In gynogenesis, parthenogenetic females need the sperm of sexual males of the same species to activate their unreduced eggs, but here also the male genome is excluded from the progeny (Bell 1982). Gynogenesis is known to occur in many animal taxa, for example amphibians *Ambystoma platineum* and *A. tremblayi*, psychid moths in genus *Luffia,* and beetles in genus *Ips* (Bell 1982, Kirkendall 1990, Løyning & Kirkendall 1996). Gynogenetic and hybridogenetic species can be considered sexual parasites (Bell 1982).

Parthenogenesis can be divided to thelytoky, arrhenotoky and deuterotoky, according to the sex of the offspring developed from the unfertilised eggs (Maynard Smith 1978, Bell 1982). In arrhenotoky, haploid eggs are laid by females that undergo meiosis. The eggs develop with or without fertilization. If the eggs are fertilized they develop into diploid females. If they are not fertilized, they develop into haploid males. In thelytokous parthenogenesis instead, only females are produced from unfertilised eggs. In deuterotoky, both females and males are produced from undersized eggs. Arrhenotoky is common among haplo-diploid hymenopterans and rotifers, while thelytoky seems to occur disproportionately often among weevils (Coleoptera: Curculionidae), chrinomid flies (Diptera: Chironomidae) and bag worm moths (Lepidoptera: Psychidae) (Suomalainen 1962, 1973, Bell 1982).

Forms of parthenogenesis can also be classified according to the presence or absence of meiotic cell divisions and according to the mechanisms by which the diploid (or tetraploid) state of offspring is retained. In automixis, haploid cells are produced meiotically from the same diploid genome (Suomalainen 1962, Maynard Smith 1978, Bell 1982), and diploidy is restored by fusion of the two haploid cells produced by meiosis, or of the two genetically identical nuclei produced by mitosis (Maynard Smith 1978, 1998). Thus, offspring produced by automictic parthenogenesis generally have lower heterozygosity than the parent but not all offspring are identical to each other (Suomalainen 1962, Maynard Smith 1978, 1998). In endomitosis, meiosis is also retained but the number of chromosomes is first doubled into the tetraploid state (to retain the diploid state of produced offspring) before the meitotic divisions. (Maynard Smith 1998, Bell 1982). Pairing then occurs between sister chromosomes. Offspring are thus identical to the parent (Maynard Smith 1998). In apomixis, meiosis is totally suppressed and here the produced offspring are also genetically identical to the parent (Suomalainen 1962, Bell1982, Maynard Smith 1998). Endomitosis is the form of parthenogenesis in certain lizard families, such as *Lacerta* and *Cnemidophorus* (Bell 1982, Maynard Smith 1998). Apomixis is the most common type of parthenogenesis and it occurs among, for example, Orthoptera, Diptera and Coleoptera (Suomalainen 1962, Bell 1982). Automictic parthenogenesis is not as common as apomixis but it is relatively common among insects, and especially among solenobid bag worm moths (Lepidoptera: Psychidae) (Bell 1982, Suomalainen 1962).

Geographical parthenogenesis refers to a situation where closely related sexual and parthenogenetic species, or sexual and asexual "races" of one species, differ in their distribution areas, often due to some environmental condition (Seiler 1923, Vandel 1928, Suomalainen 1950, Glesener & Tilman 1978, see also Suomalainen 1962 and Bell 1982). Occasionally, the asexual and sexual populations are purely allopatric but, more often, there is at least partial overlap in their distribution. Often parthenogenetic species or races occur in higher latitudes, have wider a distribution area, or occur in the peripheral or isolated parts of the species´distribution area (Suomalainen 1962, 1973, Bell 1982). This may be explained by the ability of a single parthenogenetic individual to establish a new asexual population in conditions where sexual reproduction is not possible or where there are no other sexual individuals (Bell 1982).

1.1.3 Costs of sexual reproduction

Maintenance of sexual reproduction continues to puzzle evolutionary theoreticians and has raised a multitude of studies since the question was first raised by Maynard Smith (1978) and Bell (1982). Many hypotheses have been presented to explain the maintenance of sex (see e.g. Hurst & Peck 1996, Doncaster et al. 2000, Lythgoe 2000, Jokela et al. 2003). The actual problem in the maintenance of sex is well manifested by the so-called “cost of sex hypothesis”, which predicts that an asexually reproducing female could produce its offspring more effectively than a sexual competitor could because it can avoid the costs of producing males. An asexual female should be able to replace her sexual rivals if the "all else equal" assumption is true (Maynard Smith 1978). In the long term, this should lead to natural populations consisting purely of asexually reproducing females, unless there are some short-term advantages to sexual reproduction (Lively 1996).

Sexual reproduction can suffer also from additional costs of sex. These include the well-known difficulties of attracting and finding a suitable mate that often increase the risk of predation and parasites (Sahaluk & Belwood 1984, Wertheim et al. 2003, Wing 1988, Rowe 1994, Vencl et al. 1994, Acharya 1995, Kotiaho et al. 1998). Furthermore, mating can be difficult due to variation in environmental conditions that may cause short-lived individuals to emerge at different times, if abiotic conditions vary across the population, or all individuals to be of the same sex by chance. Among closely related sexuals and asexuals, this can lead to geographical parthenogenesis (Seiler 1923, Vandel 1928, Suomalainen 1950, Glesener & Tilman 1978, see 1.1.3 Parthenogenetic reproduction). The increased risk of sexually transmitted diseases (Bourtzis & O´Neill 1998) and the costs of copulating (Arnqvist 1989, Rowe 1994, Rice 1996, Blanckenhorn et al. 2002) are also costs of sex. In small populations, sexually reproducing species may be subject to inbreeding depression (Milner-Gulland et al. 2003). The only chance for mating may be with closely related individuals or with an individual whose reproductive value is reduced by previous matings (see e.g. Kirkendall 1990). On the other hand, mating with an other individual (especially with a migrant) may cause a break up of gene complexes that would be favourable in this particular environment (Peck et al. 1998, Maynard Smith 1978, Greig et al. 1998, Ahlroth et al. 2003). Furthermore, sexual reproduction can be subject to reproductive parasitism by hybridogenetic and gynogenetic asexuals (see e.g. Bell 1982, Kirkendall 1990, Løyning & Kirkendall 1996). Additional costs of sex have been found in several butterfly species, where multiple mating by males leads to a reduction in spermathore size, i.e. the amount of nutrients that males provide females during copulation, (Watanabe et al. 1998, Rutowski et al 1987). Similarly, the finite number of sperm per male can lead to additional costs of sex due to multiple mating of males (see Wedell et al. 2002). This can cause a significant drop in the proportion of hatched eggs and cause significant fitness costs for females. In many marine systems, sperm limitation is common and various reproductive strategies (e.g. large egg size) have evolved to minimize the females´ risk of remaining unfertilised (e.g. Levitan 1998).

1.1.4 Advantages and maintenance of sexual reproduction

With the apparent costs of producing males by sexual reproduction, it is essential to ask what keeps sexual reproduction hanging on. Williams (1975), argued that there must be some immediate advantage to sexual reproduction to counterbalance its twofold disadvantage. Many hypotheses have been presented to explain the maintenance of sex since it was first raised by Maynard Smith (1978) and Bell (1982) (see e.g. Hurst & Peck 1996, Doncaster et al. 2000, Lythgoe 2000). None of these, however, has yet reached common acceptance by all scientists.

One of the most remarkable of these hypotheses is the mutation accumulation hypothesis. This is based on the knowledge of the accumulation of harmful mutations in asexually reproducing species in each generation. Most mutations are harmful and if a species is unable to recombine its genome the only way to get rid of harmful mutations would be a reversal of the harmful mutation (Maynard Smith 1998). As this is an unlikely event, asexuals are subject to accumulation of harmful mutations while sexually reproducing species are able to recombine their genome in cross-fertilisation which purges deleterious mutations and increases the amount of genetic variation (Muller 1964, Kondrashov 1988, Rice 2002). With a considerable rate of mutations per genome (Keightley et al. 2000), mutation accumulation in asexuals could explain why sexuals can outnumber them (Lively 1996, Peck & Waxman 2000, Rice 2002).

Most other hypotheses put forward to explain the persistence of sex are linked with the abilities of sexual reproduction to create and spread advantageous traits (Hurst & Peck 1996). Sex can speed up evolution because in sex beneficial mutations that have evolved in different individuals can be brought into the same individuals (Maynard Smith 1978, 1998). In asexually reproducing species such mutations would have to occur in same line as genetic recombination is not possible between individuals (Bell 1982, Maynard Smith 1978, 1998).

Sexual reproduction not only enhances the speed of evolution. Remarkably, it also vastly increases the genetic variability of offspring (see Maynard Smith 1978, Hamilton 1980). It is also the birth of rare genotypes that could give sexual reproduction a decisive advantage over asexual reproduction in competitive interactions. The importance of this variation in host genotype is further developed in the "parasite hypothesis" (Hamilton 1980), which emphasises the effects of co-evolution between host and parasite. Co-evolutionary interactions, together with strong frequency dependent selection by high parasite prevalence, could favour variability in host genotypes. Since parasites most likely track the most common genotypes, rare genotypes might avoid infections (Hamilton 1980, Fox et al. 1996, Howard & Lively 1998, Ooi & Yahara 1999, Lively & Dybdahl 2000). This co-evolution can lead to continuous changes in the frequency of host genotypes. This idea is manifested in the recently popular Red Queen hypothesis (Van Valen 1973) and in the parasite hypothesis (Hamilton 1980). Recent support for the parasite hypothesis for sex was presented by Negovetic & Jokela (2001) who found that asexual snails originally reproduced more successfully than the sexuals, but in unfavourable environmental conditions, the situation was reversed. The parasite hypothesis has also been supported by studies on at least two different freshwater snail species (Lively 1987, 1992 and Johnson 2000), bivalves (Grosholz 1994), flatworms (Michiels et al. 2001), geckos (Moritz et al. 1991), fish (Lively et al. 1990) and yeast (Greig et al. 1998).

Sexuals´ higher recombination rate should make them more resistant to parasites than asexuals, unless the number of asexual clones is high enough to account for an equal amount of genetic variation in the asexual population. Occasionally, however, sexual populations can suffer from genetic problems (such as inbreeding and low heterozygosity levels) (see e.g. Milner-Gulland et al. 2003). These can cause lowered parasite resistance among sexuals and an asexual population might then be better able to successfully coexist with such a sexual population. Sexual populations may loose genetic variability and particularly the rare genotypes that most likely survive the parasites (see e.g. Hamilton 1980, Jokela et al. 2003).

Some studies have focused on the mechanisms restricting the success of facultative parthenogenesis within species capable of both sexual and also asexual reproduction, with the latter often functioning as a purely secondary reproductive strategy in the absence of a suitable mate (e.g. Corley et al. 2001). Theoretical studies have also focused on co-existing sexuals and asexuals (e.g. Rispe & Pierre 1998, Peck & Waxman 2000), but the actual problem of the maintenance of sex still remains and there is a lack of empirical data needed to determine the question. Apart from the outstanding studies on New Zealand freshwater snails (Lively 1992, Fox et al. 1996, Jokela et al. 1997, Howard & Lively 1998), there is virtually no empirical data about natural systems where sexually and asexually reproducing morphs of non-hybrid origin simultaneously coexist and whose two morphs would compete with each other in a common natural environment (see however Johnson 2000, Michiels et al. 2001).

Data is even more limited on natural systems where the effects of parasites on their hosts are severe. Studies with less harmful parasites have been made (e.g. Hanley et al. 1995, Michiels et al. 2001). Still many authors have argued that it could be the parasites or pathogens in particular causing serious reduction in the fitness of their hosts. It is this, they argue together with moderate rates of mutation that most likely favours sexual reproduction (e.g. Howard & Lively 1994, West et al. 1999). In many arthropod species asexuality is not a true reproductive strategy but caused by a feminising bacteria, which can increase the mortality of male embryos. Some strains of *Wolbachia* bacteria force genotypic males to act as phenotypic females and drive sexual females to reproduce parthenogenetically (Howard & Lively 1994). These effects may, however, be considered true costs of producing males as asexually reproducing morphs rarely carry the harmful effects of these bacteria infections (Bourtzis & O´Neill 1998, Kageyama et al. 1998). Thus, more empirical data are needed to support any of the hypotheses around the maintenance of sex.

1.2 Ecological and environmental factors

Occasionally, the proportions of sexual and asexual reproduction among different populations of a species, or among closely related sexual and asexual species, may be connected with environmental conditions that vary across geographical axis. Among the most well-known examples are the distantly allopatric distribution areas of weevil *Otiorrhyncus dubius* (Coleoptera: Curculionidae), which occurs as a diploid sexual race in Central European mountains and as a tetraploid parthenogenetic race in a separate distribution area in northern Europe (Suomalainen 1969). Such distinct and distant distribution of parthenogenesis is often referred to as geographical parthenogenesis (Seiler 1923, Vandel 1928, Suomalainen 1950, Glesener & Tilman 1978, see also Suomalainen 1962 and Bell 1982). More often closely related sexuals and asexuals have at least partially overlapping distributions. On a minor geographic scale, certain habitat conditions, e.g. temperatures, general climate or current phase of vegetational succession, can also facilitate either sexual or asexual reproduction (see Bell 1982). For some species, successful sexual reproduction may require good weather, a warm microclimate or dense population. If these vary between locations, the proportions of sex and asex may vary even between neighbouring populations due to difficulties in finding or attracting a mate in suboptimal conditions.

Certain ecological factors may reduce the success of sexual reproduction. Sexuality may suffer from the predation for example. Predation of individuals searching for or attracting a mate was previously considered an additional cost of sex (see e.g. Lloyd & Wing 1983, Sahaluk & Belwood 1984, Andersson et al. 1998). If predation is more severe against sexuals, it could favour asexual reproduction in certain habitats. However, ecological factors have seldom been taken account in studies of maintenance of sex.

1.3 Aims of the study

This thesis is based on studies of phylogeny, the evolutionary history of reproductive strategies, occurrence of sexual and asexual reproduction, cost of sex, effects of hymenopteran parasitoids, additional costs of sexual reproduction, genetic variation among populations and the effects of wood ant predation in solenobid psychid moths (Lepidoptera: Psychidae). The first paper is a study of phylogeny and the evolution of sexual and asexual reproduction in solenobids using mitochondrial DNA to construct phylogenetic trees. I compared these trees to the current relationships of the species, which are based on their morphological features. I also aimed to study the origin of parthenogenesis in these species. In paper II, I compared the life history traits of the sexual *S. rupicolella* and asexual *D. fennicella* species to test for cost of sex. I then tested the possibility of *Wolbachia* bacteria causing parthenogenesis in these moths. Finally, I studied the occurrence of sexual and asexual reproduction and parasitoid prevalence in different study populations to see if parasites could favour sexual reproduction. In study III, I investigated the additional costs of sexual reproduction by assessing males´ ability for multiple mating. I compared the proportion of hatched offspring produced by the first, second and third mating of the same males, in the two most common sexual solenobid species. In study IV, cellulose acetate electrophoresis was used to compare the amount of genetic variation in sexual and asexual bag worm moth populations and to assess the gene flow between different moth populations. The final paper (V), tests for the direct and indirect effects of ant predation on the occurrence of solenobid bag worm moths. First, I compared the abundance of ants and bag worm moths between populations. Secondly, I compared the abundance of ants, bag worm moths, the natural enemies of bag worm moths and other important taxa between trees with and trees without wood ants (*Formica rufa* group). Finally, I studied the direct effects of ants on parasitoids and moth larvae in three different sets of ant behavioural experiments.

2 STUDY SYSTEM

In Finland, seven species of solenobid moths are known to occur (Suomalainen 1980). Solenobids (Lepidoptera: Psychidae) contain two genera: *Siederia* and *Dahlica*, among which the Finnish species are both morphologically and ecologically uniform. Finnish solenobids include the species *Dahlica triquetrella, Dahlica lichenella, Dahlica fennicella*, *Dahlica lazuri*, *Dahlica charlottae*, *Siederia listerella* and *Siederia rupicolella*. The status of many solenobid species is still uncertain and species determination is mainly based on a few morphological features, such as the shape of the scales on male front wings and the length of the minor spines on male front legs (see Suomalainen 1980, Hättenschwiler 1997, study I). Among the Finnish solenobid species, at least three separate parthenogenetic species have been identified (see Suomalainen 1980). In Finland, solenobids occur mostly in older wooded habitats, especially in sparse forests or in warmer forest edges. In Central Finland, these habitats are patchily distributed and are often occupied by several species. Asexual *Dahlica fennicella* and sexual *Siederia rupicolella* are the most common species in most of the suitable forest habitats of Central Finland.

The life cycle of solenobid bag worm moths from egg to adult takes from one to two years. However, the adult stage takes only 3-6 days (Suomalainen 1980). Bag worm moth larvae are characterised by a larval case (or sack), which is made of fragments of plant material, sand and other dry matter. All larvae carry a larval case throughout their long larval development. The larvae of solenobid bag worm moths feed on moss and algae that grow at the base of tree trunks, on large exposed tree roots and on rocks and stones. Larvae climb tree trunks for each moulding and fully-grown larvae climb tree trunks or rocks to pupate within their larval case. Adult females of all *Dahlica* and *Siederia* species are wingless, sessile and incapable of dispersal. Once hatched, sexual females excrete pheromones to attract males. After copulation, females lay their eggs in their own larval case and die within few hours. Parthenogenetic females, instead, do not excrete pheromones but start to lay eggs shortly after hatching from pupa. Males are always winged and search for newly hatched females, guided by female pheromones, but males have a very limited dispersing ability.

They can only fly short distances (between 10 and 100 m). The length of adult females is 3-5 mm on average and the wingspan of males is 13-16 mm on average. Larvae size is equal to that of adult female and the larval case averages 6-8.5 mm. (see Suomalainen 1980, Hättenschwiler 1997)

Psychid larvae are often infected by two common species of Hymenopteran parasitoids (see Hättenschwiler 1997, also Hättenschwiler pers. comm.) e.g. *Orthizema* spp. (Hymenoptera: Ichneumonidae). Parasitoid females normally lay one egg per host larva and the parasitoid eggs remain dormant until the fully-grown host larvae pupate. The parasitoid larva then quickly eats the host, pupates and hatches. Thus, the cost of being parasitised is extreme. In preliminary laboratory experiments, I offered different sized larvae of several solenobid species to females of the most common parasitoid species. The parasitoids did not discriminate between host larvae of different *Dahlica* or *Siederia* species or between larvae of different size, as long as the larvae were fully-grown (i.e. the larval case was at least 6 mm long). Similarly, Thomas & Elmes (1993) have found that parasitoids infecting a lycaenid butterfly reject small host larvae (less than 14 mg) while large host larvae (more than 16 mg) are infected by the parasitoid.

Based on previous observations, I knew that both the parasitoid prevalence and the proportions of sexual and asexual moths vary strongly between the study locations. *S. rupicolella* (sexual) and *D. fennicella* (asexual) were chosen as the main study species because these two are locally the most common species (80% or more of all species of Psychidae moths).

Psychid moths are not known to posses effective anti-predatory features such as spines, hairs, hard exoskeleton, good escaping abilities, poison or unpalatability (see e.g. Hättenschwiler 1997). Solenobid psychid larvae carry a thin-walled, soft larval case made of silky thread and covered by various small particles of organic and inorganic origin. The larval case is unlikely to protect larva from predators. This view is supported by my field observations of bugs and coleopterans that enter the larval case from its opening end and predate on the larvae (pers. obs.). Given this lack of protection, combined with the very limited dispersal abilities of bag worm moths both as larva and adult, it is surprising that I found psychid moths to be numerous in the warmer edges of older forests where abundant wood ant colonies, *Formica rufa* group (Hymenoptera: Formicidae) hunt. Furthermore, within these habitats, ant nests are often situated very close to the trees where the bag worm moth larvae climb and thus ants are often locally very abundant on these tree trunks and on the ground close to "bag worm moth trees". Wood ants are aggressive predators (see e.g. Collingwood 1979, Skinner & Whittaker 1981, Hölldobler & Wilson 1990, Halaj et al. 1997) that hunt on tree trunks, and forage on other invertebrates (e.g. Fowler & MacGarvin 1985, Haemig 1994, Aho et al. 1997, 1999). They are known to alter the distribution and abundance of many arthropods including Lepidoptera larvae (Laine & Niemelä 1980, Skinner & Whittaker 1981, Karhu 1998).

Psychid moth larvae along with most Lepidoptera larvae, can be regarded as ideal ant prey animals: they are easy to catch and often occur in large numbers. My own field observations of ant predation indicate that wood ants affect most arthropod species coexisting with ant colonies severely. Lepidoptera larvae living and climbing at and near the base (lowest 2-3 metres) of trees trunks are particularly negatively affected. The co-occurrence of wood ants and solenobid bag worm moths is a previously undetected phenomenon and, to my knowledge, no studies have been made about the co-existence of these two and certainly not about possible effects of ants on bag worm moths. Wood ants are also likely to reduce the number of hymenopteran parasites and predators of psychid moths. If ants do not hunt psychid moths, while they predate on the parasitoids that infect psychid moths, they might even favour asexual reproduction of the psychids. This would be due to reduced parasitism by the hymenopterans, which are expected to favour sexual reproduction among their hosts (see 4.1.2 Advantages and Maintenance of sexual reproduction).

3 MATERIALS AND METHODS

In early spring, larvae of *Siederia* and *Dahlica* species climb up tree trunks, rocks and walls for pupation, as snow usually still covers most of the ground at this time of year. Bag worm moth larvae can be easily collected by setting tape traps on tree trunks before the larval "climbing period" (30 March to 7 April). Climbing larvae stick to tape traps and can be detached using tweezers without harming the larvae. I set the traps and checked them twice per week until the "climbing period" was over (30 April). The samples for all studies (I, II, III, IV and V) were collected mostly from the tree trunks of Norway spruce (*Picea abies*) and silver birch (*Betula pendula* Roth) and in one population (Rauma) also by searching for larvae on the shadow and undersides of larger stones. After collection, the larvae were individually canned, labelled and raised until adulthood in the laboratory. The main study areas (for studies I, II, III, IV and V) were situated in Jyväskylä, Central Finland (62 °15 N', 25°43 E').

3.1 Sexual and asexual reproduction

3.1.1 Phylogenetic occurrence of asexual reproduction in psychid moths

All Finnish samples for phylogenetic studies, except *D. lichenella*, were collected from natural populations of bag worm moths around Jyväskylä (see above). *D. lichenella* samples were collected from Rauma (61° 06' N, 21° 34' E) in south-western Finland. Additional samples of *S. rupicolella* were collected from Orimattila (60° 48' N, 25° 44' E) in southern Finland and from Sonkajärvi (63° 39' N, 27° 28' E) in the northern part of central Finland. Additional samples of *D. triquetrella* were collected from Lapinlahti (63° 22' N, 27° 24' E), in the northern part of central Finland. All Finnish samples were collected in spring 2001 and spring 2002. Samples from Switzerland and British Columbia, Canada were obtained from other collectors.

Males were used for the analysis of all sexual species except *S. rupicolella,* for which sexual females were also used. Males were classified into different species based on the wing scales, whereas mate choice and acceptance were used to reliably determine the species of sexual females. For parthenogenetic species the form of the larval case and the distribution area were used as classification methods. The outgroup species, *Diplodoma laichartingella* and *Psyche norvegica,* were determined by the shape, material and size of their larval case. The samples were preserved either at -20 °C or in absolute ethanol until analysis. Total genomic DNA was extracted from the entire individual using a chelating resin (Pearce et al. 1997). A fragment of about 600 bp spanning from the end of the COI to the COII and another of about 400 bp of the 12S rRNA gene were amplified by PCR and used for the phylogenetic analysis. Phylogenetic trees were obtained with three different methods: Maximum Parsimony (MP), Neighbour Joining (NJ) and Bayesian analysis.

3.1.2 Maintenance of sexual reproduction

To study the cost of sex, larvae of sexual and asexual solenobids were collected from two locations, in Jyväskylä, (Sippulanniemi 1 and Huviniemi), in spring 1999. A total of 211 tape traps were set in the two study populations (138 and 83 respectively). Larvae were collected as described above (3. Materials and methods) and were raised to maturity in the laboratory. Adults of sexual *S. rupicolella* were paired with randomly assigned mates while females of parthenogenetic *D. fennicella* reproduced without intervention (for a more detailed description of the mating system see 3.1.3 The additional cost of sexual reproduction). Life-history and morphological features were compared between sexual *S. rupicolella* and asexual *D. fennicella.*

To exclude the possibility that parthenogenesis in bag worm moths is caused by *Wolbachia pipientis,* 85 females (44 parthenogenetic *D. fennicella* and 41 *sexual S. rupicolella*) and 10 parasitoids were tested for the presence of this bacteria with PCR. The samples for this analysis were randomly collected from several study populations.

Parasitoid prevalence and the proportion of sexual reproduction in psychids in 1999 were determined in 15 different study locations within a relatively small area (15 km x 30 km) around Jyväskylä. This allowed us to study the effects of parasitoids on the occurrence of sex. In the following two years, 2000 and 2001, parasitoid prevalence and the proportion of sexual reproduction were determined in 29 and 19 locations respectively. In 2000, additional areas in southern Finland, near Orimattila, and in the northern part of Central Finland, around Sonkajärvi were sampled. In each area, 5 to 138 tape traps were set. In smaller forest patches, almost all trees were tape trapped whereas in larger forest patches trunks were chosen randomly

In the laboratory, the reproductive mode and sex of the hatching moths were monitored. Reproductive strategy results are based on moth individuals not infected by parasitoids as infected individuals are always lost and thus neither their reproductive strategy nor their species can be determined.

Approximately four weeks after the adult moths´ hatching period (two months after collecting the larvae), parasitoids began to hatch from the moth pupa, infected in the field. The parasitoid prevalence in all locations was determined and compared to the proportions of sexually and asexually reproducing moths in each location. The parasitoid prevalence is intended as the total parasitoid load and thus covers all the Hymenopteran parasitoids hatching from moths. Populations where parasitoid prevalence was 100 % were excluded from further analysis because there the proportions of reproductive modes could not be determined. I also analysed the effects of habitat characteristics (size, location, and main tree species) on the proportion of sexual reproduction in each.

3.1.3 Additional costs of sexual reproduction

The sex ratios of *S. rupicolella* and *D. charlottae* were studied in spring 1999 and spring 2001 in 27 separate populations. Samples for laboratory studies were obtained by setting tape traps as described above. Collected larvae were taken to the laboratory where they were raised into adults. The species was determined for all hatched adults (see I) and the sex ratios for both species were calculated. Populations were excluded from sex ratio studies if the parasite prevalence was 100 % (and sex ratios could not be determined) or if the number of adults was less than ten individuals (and sex ratio could not be reliably determined).

I collected individuals for multiple mating experiments and individuals for sex ratio studies at the same time. After hatching from pupa, females climbed on the top of their own larval case and started excreting pheromones and waiting for males to arrive. At this point, I offered each female a randomly chosen male and let them copulate. Males quickly began flying towards the females. Copulation normally succeeded immediately and lasted between 5 and 30 minutes. After copulation, females laid their eggs inside their own larval case. Females never waited for another copula or for another male and died within a few hours of egg laying. This suggests that females are able to copulate and reproduce only once. If a male was still able to fly after the first copula, he was offered a chance to copulate with a second and third (in 1999 also with a fourth) female (one exceptional male of *D. charlottae* had five mates) and these copulations were facilitated as noted above. The effects of the length of male recovery period on larval hatching success were tested. Egg clutches laid by individual females were kept separate. The number and proportion of hatching larvae were calculated and larval hatching success was compared between the first, second and third matings. Also the effects of the origin of male and female were tested on the proportion of hatched larvae.

3.1.4 Role of genetic variability

To study the genetic variability of sexual and asexual solenobid species, three species were selected on the basis of their abundance in Central Finland and the facility with which they could be classified. The studied species include two sexual species, *Siederia rupicolella* and *Dahlica charlottae,* and an asexual species D. *fennicella*. Samples were collected from 20 different study areas in Central Finland. Collected samples were frozen until analysis. Samples were run on cellulose acetate plates (Helena laboratories) following the method of Hebert and Beaton (1993). Ten enzymes representing thirteen loci were scored. The genetic diversity and population structure of the sexual species were analysed using the software package Fstat (Goudet 1995). Because of the type of parthenogenesis and the lack of recombination in the females of this species, individuals most likely show fixed heterozygosity because of no disjunction of alleles. In consequence, genotypes, rather than allele frequencies, should be considered (Samadi et al. 1999) and Fst statistic cannot be conducted (reviewed in Rousset & Raymond 1997). Clonal diversity within populations was determined simply as the proportion of different genotypes in the population: $k = G/N$, where G is number of genotypes and N is the number of individuals in the populations. Comparison of genotypic frequencies among asexual populations was carried out using a log-likelihood (G) based exact test using Genepop (Raymond & Rousset 1995). Only one copy of identical genotypes per populations was retained in the analysis (Sunnucks et al. 1997).

3.2 Ant predation

3.2.1 Effects of ant predation on psychid moth populations

To study if wood ants (*Formica rufa* group) affect the occurrence of psychid moths either by predating on psychid larvae or by reducing the number of their enemies, I measured both their direct and indirect effects on psychid moths and parasitoids by 1. assessing which environmental factors, including ant numbers, affect the local abundance of bag worm moths, 2. comparing moth, parasitoid and general arthropod numbers on ant and non-ant trees and 3. testing if moth larvae are considered prey by ants.

3.2.1.1 Habitat characteristics

To investigate which habitat characteristics have an effect on bag worm moth abundance, I measured habitat size, forest age, main tree species, coverage (in terms of percentage) of the main vegetation layers (mosses, ground layer, tree top), temperature variation, vascular plant and moss species diversity, lichen abundance and ant abundance in 38 study locations. I set a max-min thermometer in each location to measure maximum and minimum temperatures. The thermometers were checked for the highest and lowest recorded temperatures at the end of each month between August 2001 and August 2002. Average temperatures and standard errors of average temperatures were determined for all study locations for the entire study period (13 months).

In each study location, I counted the number of wood ant nests (Hymenoptera: Formicidae: *Formica rufa* group) and ant paths. Similarly, I counted ant traffic on five randomly selected spruce trunks (*Picea abies*) or, if no spruce were available, on birch (*Betula pendula)* in each location. The average ant traffic for each locality was calculated on the basis of these five trees per location. I determined an additional ant activity value (between 1-5) for all locations by assessing the average ant activity at the edge areas of the forest patches.

Principal component analyses were performed separately for ant associated and forest descriptive variables.

3.2.1.2 Ant trees versus non-ant trees

Because locations differ in many characteristics, correlative comparisons can give confounding results. Thus, I studied the direct effects of ant predation on psychid moths and their natural enemies, and on other relevant arthropod taxa within locations. Tape traps were set on 36 pairs of tree trunks in an area of 16 km x 8 km, in the vicinity of Jyväskylä. This area included most habitat types favoured by bag worm moths in which *F. rufa* colonies were abundant. Within a pair of trees, there was always a tree with heavy ant traffic and another tree without or with only minor ant traffic. The traps were set in the beginning of August 2001 and were checked twice every month, until October 2001. The traps were reset in April 2002 and checked until the end of October 2002. Tape traps collected most of the arthropods that climbed (or rested) on tree trunks, excluding ants. At each check, I identified, counted, and removed all trapped arthropods. A reliable ”ant traffic” value for "ant trees" and "non-ant trees" was obtained by calculation, independent of the ant traffic value for populations (see previous paragraph). The collected arthropods were classified into the following groups: small psychid larvae, large psychid larvae, ant prey animal (including non-psychid Lepidoptera larvae, adult Diptera, Orthoptera, chrysomelid beetles, Trichoptera and all other Hymenoptera except ants and parasitoids that infect psychid moths) and predatory arthropods (spiders, harvest-spiders and predatory Coleoptera; e.g. carabids and staphylinids). The number of individuals for each group was tallied from the whole collecting period. The total numbers were compared between ant trees and non-ant trees.

3.2.1.3 The reactions of ants

I tested the direct reactions of ants (*Formica rufa* group) to psychid moth larvae and hymenopteran parasitoids. In three separate experiments, I facilitated encounters between ants and potential prey items, on ant paths (on the ground). The reactions of ants towards the prey items were observed over a period of five minutes, or until one of the prey items was taken by the ants. The reactions of ants were categorised into five different degrees of aggression: 0 = no reaction to encountered item, 1 = ant stopped at encountered item, 2 = ant briefly tried to lift the prey item, 3 = ant spent more than one second pulling the prey item in order to detach it from the tape, 4 = ant took the prey item. After the experiment, mean degrees of ant aggression towards different prey items were calculated and these values were compared between the prey items. In the first experiment in June 2001, the aggressiveness of the reactions of wood ants to psychid moth larvae was studied in four separate wood ant colonies in a total of 27 repeats. Wood ants´ reactions to solenobid larvae were compared to their reactions to a potentially interesting prey item (mosquito) and a potentially less interesting prey item (spruce needle). In the second experiment in August 2001, I compared the reactions of ants towards *D. fennicella* and *S. rupicolella* larvae with and without the larval case. In the third experiment in July 2002, the ants´ reactions to hymenopteran parasitoids (*Orthizema* spp.) were compared to their reactions to the larvae of *D. fennicella* and *S. rupicolella* within their larval case.

4 RESULTS AND DISCUSSION

4.1 Sexual and asexual reproduction

4.1.1 Phylogenetic occurrence of asexual reproduction in psychid moths (I)

In study I, the reliability of species determination based on wing scale morphology was evaluated by comparing the morphological classification with a phylogenetic tree obtained using mtDNA. The species determination based on morphological characteristics did not correspond to the one based on mtDNA markers. These results indicate that species determination based solely on morphological characteristics, such as wing scales, is not reliable in this confusing group of species. However, at least in Finland, where the number of species is relatively low, some species can easily be determined also based on their wing scale morphology. In areas with more solenobid species, several morphological features together with ecological and distributional data are needed to reliably determine the sexual species. A few sexual species, and probably most asexual solenobids, would need even closer analysis, possibly including genetic analysis.

Dahlica and *Siederia* species did not cluster on clearly separate branches and thus, on the basis of the molecular phylogeny, the current status of these two genera is questionable. Further analysis is needed to determine this question. Other closely related species, such as species in genus *Postsolenobia,* should be included in the analysis (see Sauter & Hättenschwiler 1991, Hättenschwiler 1997).

It is apparent that wing scale morphology has led to the misidentification of many specimens of *Dahlica lazuri* and sexual *Siederia,* indicating that intraspecific variation for this character is higher than previously suggested. In the case of parthenogenetic species, ecological and distributional data are helpful for species determination in Finland. However, because of the high number of different species in Central Europe, it is questionable if ecological or distributional data are generally useful. My results indicate at least three separate origins of parthenogenesis in *Dahlica* species. Thus asexual reproduction might be favoured in this group because several asexual species remain instead of going extinct. This study has also raised many new questions and revealed novel problems. Firstly, my results with mtDNA markers question the relevance of two separate genera. Secondly, the high genetic differentiation for certain species and the incongruence between morphological characters and mtDNA sequences suggest that several samples from different populations should be analysed to elucidate the relationships among psychid moths.

4.1.2 Maintenance of sexual reproduction (II)

In study II, I compared the life-history traits of sexual *S. rupicolella* and asexual *D. fennicella* in two locations where these two species co-occur and compete for the same resources and are often infected by the same Hymenopteran parasitoids. The purpose of this study was: 1. To test predictions of the cost of sex hypothesis, and 2. To find out if strong selective pressures, such as high parasitoid prevalence, could favour sexually reproducing species. This is the basic assumption of the "parasite hypothesis for sex" (see e.g. Hamilton 1980, Jokela et al. 2003). The two species are morphologically and behaviourally extremely similar and can be separated only by their reproductive strategy (sexual vs. asexual) or by genetic markers. In this study, I could find no difference in female size, number of larvae or offspring survival between sexuals and asexuals. Because it takes two to reproduce sexually and only one to reproduce parthenogenetically, the sexual species should produce twice the amount of offspring as its asexual competitor. As this was not the case, these results indeed indicate that sexuals are subject to the two-fold cost of sex.

If the sexual moths bear this two-fold cost of reproducing sexually, how are they able to persist with their asexual rivals? - It seems that asexuality is not caused by *Wolbachia* bacteria. None of the samples were infected by *Wolbachia* and, thus, it is unlikely that these bacteria have affected my results. But, when I sampled 38 locations to study the prevalence of parasitoids and the proportions of sexually and asexually reproducing psychid moths in each population, I found a strong positive correlation between prevalence of sexual reproduction and prevalence of parasitoids. This result means that in locations where parasitoids are rare, asexuals exist in high densities; whereas in locations with a high parasitoid load, the sexual species was dominant. Spatial distribution of sexuality or asexuality alone does not explain these results. I suggest that the "parasite hypothesis for sex" may explain the persistence of sexual moths in this system. This would mean that coevolutionary interactions between host and parasite (Hamilton 1980) and, particularly, the role of genetic variability in increasing resistance to the parasite favour sexual reproduction (see e.g. Grosholz 1994). According to the parasite hypothesis, co-evolutionary interactions between host and parasite, together with strong frequency dependent selection caused by the parasites, could favour variability in host genotypes. This is because parasites are most likely to track the most common genotypes and the rarer genotypes may avoid parasite infection (see Hamilton

1980). Thus, the occurrence of common parasites could favour sexuals with higher recombination rate unless the number of asexual clones is very high (for discussion of the hypotheses, see Hamilton 1980, Lively 1992, 1996, Greig et al. 1998, Jokela et al. 2003). Alternative explanations for the persistence of sex include mutation accumulation and the geographical hypothesis. Neither of these are supported by the results of this study because: 1) The sexual and asexual species overlap in their distributions and also habitat size had no effect on reproductive strategy and, 2) It is unlikely that mutation accumulation could explain the maintenance of sexuals as the life-history measurements do not indicate any phenotypic abnormalities among asexuals.

Results of this study suggest a cost of sex among sexually reproducing *S. rupicolella.* Furthermore, the results suggest that a strong selective pressure caused by high parasitoid prevalence could indeed favour sexually reproducing bag worm moths instead of asexuals, as assumed by the "parasite hypothesis for sex"

4.1.3 Additional costs of sexual reproduction (III)

In study III, I found multiple mating of males to significantly decrease the fertility success of females of both study species. These results were not affected by the origin of either males or females. The longer recovery period of male after the first mating did not result in higher fertilisation success. Multiple mating of males caused a drop in the proportion of hatched eggs in both species causing significant fitness costs for the females. This reduction was clear in both species and in both study years. The number of hatched larvae decreased with the increasing number of copulations for males in both species. When mated with already mated males *S. rupicolella* females produced 59% and 29% less larvae than females that mated with virgin males in 1999 and 2000, respectively. In the same years, females mated with males that had already mated twice, produced respectively 100% and 44% less larvae than the females mated with the virgin males. *D. charlottae* females suffered a 24% loss in the number of produced larvae when mated with already mated males in both years. When mated with males that had already mated twice, females suffered a 29% and 44% reduction in the number of produced larvae, in 1999 and 2000 respectively. Because psychid moth females are not known to obtain spermatophore or other energy rich resources from the males to improve their survival and reproductive output, this reduction in fertilising ability is most likely due to the insufficiency of male sperm in subsequent copulations. Even a small reduction in fertilising ability can be fatal for female solenobids as many populations are small, some populations are female biased and females seem unable to reject a lower-quality mate. With the reduced dispersal abilities of males and total winglessness of females, the sperm shortage is likely to occur in natural populations. I suggest that these results together with biased functional sex ratio imply additional costs of sexual reproduction for sexual species, which have to compete with closely related asexual species in which reproduction is not limited by the availability of males. These results imply that sexual bag worm moths, and *S. rupicolella* in particular, are subject to more than just two-fold cost of sex (II).

4.1.4 Role of genetic variability (IV)

In study IV, I investigated the genetic variability of sexual and parthenogenetic bag worm moth species in Central Finland populations, using allozymes variation. In both sexual species, I found heterozygosity deficiencies and lower heterozygosity levels compared to asexual moth populations. These results suggest that many sexual populations are small and suffer from loss of genetic variability, probably due to inbreeding depression and population subdivision. I observed large genetic differentiation between different populations. This indicates low gene flow between sexual moth populations and, thus, supports the previous knowledge of the poor dispersal ability of winged males. Genotype diversity was lower in asexual *D. fennicella* than in the sexual *S. rupicolella* and *D. charlottae*. Nevertheless, clonal diversity was high in all asexual populations. This may allow them to successfully compete with the sexual species.

I also found a clear negative relationship between average population heterozygosity and parasitoid prevalence in *D. charlottae*. The parasitoid prevalence was positively correlated with the level of inbreeding (Fis) in the populations indicating that more inbreed populations are less resistant to parasitoids. In *S. rupicolella* no such trend was observed, perhaps because the level of inbreeding (although high) was quite uniform in the different populations in contrast to *D. charlottae*, although I observed large variation in parasitoid prevalence among populations of both species.

4.2 Ant predation

4.2.1 Role of ant predation (V)

I found that untended bag worm moth larvae (Lepidoptera: Psychidae) were abundant in forest habitats where wood ants (*Formica rufa* group) were also abundant (Study V). Within habitats, trees patrolled by ants supported significantly more psychid moth larvae and significantly less larvae of other Lepidoptera. Parasitoids and predatory insects were also less abundant on these trees. I monitored ant reactions towards psychid larvae and their enemies in three different behaviour experiments and found that ants aggressively attacked parasitoids while psychid larvae, within their larval cases, remained unharmed. Larvae without the larval case were readily attacked by ants. I suggest that the case protects bag worm moth larvae from ant predation. Ants most likely do not recognise psychid moth larvae as prey when in their larval case. While ants do not tend psychid moth larvae, they indirectly promote the moths' survival by predating their natural enemies such as spiders and parasitoids.

In conclusion, the most intensively patrolled ant trees are likely to serve as relatively safe feeding and pupating sites for psychid moths because wood ants reduce the numbers of parasitoids and other arthropods. Although ants could indirectly favour asexuality among psychid moths by preventing parasitism that is known to favour sexuality, I found no effects of ants on the proportion of sexual reproduction in populations. Furthermore, ants are unlikely to pose a threat to psychid moth larvae as long as the larvae manage to remain inside their larval case. According to my knowledge, these results are the first to indicate indirect positive effects of ant predation on untended Lepidoptera larvae and on any animals that are potential, and here proved to be suitable, food for the co-existing and abundant wood ants.

5 CONCLUSIONS

This thesis reports studies of the evolution of reproductive strategies in a fascinating and poorly known system; solenobid bag worm moths (Lepidoptera: Psychidae) where strictly asexual and strictly sexual moths co-occur, compete and are affected by common selective pressures. The asexual and sexual bag worm moth species form a closely related group, where species boundaries are difficult to determine without the use of genetic markers (I).

My studies present new information about the evolution and maintenance of sex. This is one of the most puzzling questions in evolutionary ecology (Williams 1975, Maynard Smith 1978, Bell 1982). In the phylogenetic relationships of bag worm moths, I have found indications of multiple, independent origins of asexuality (I), although asexuality is known to be rare among Lepidoptera (Suomalainen 1962, Bell 1982). High frequency of parthenogenesis in this group indicates that selection favours parthenogenesis more than in other Lepidoptera due to some problems of sexuality. Such problems could include the additional costs of sexual reproduction found in the two most common sexual solenobids. The sexual species commonly live in small and isolated populations where distorted functional sex ratios were both expected and detected (III). In female-biased populations, males would have to mate more than once to inseminate all the females in the population, but I found that males of two sexual species are able to successfully fertilise the eggs of only their first mate (III). Consequently, females suffered a serious reduction in the number of produced offspring. As females are able to mate only once and no mate choice by females was detected, small sexual populations may be less competitive than their closely related asexual rivals.

The cost of sex theory predicts that sexual reproducers suffer a two fold cost compared to asexuals (Maynard Smith 1978), because sexuals also produce males. In my study, I found no indications of differentiated life-history traits between the most common asexual and sexual species (II). Thus, the "all else equal" assumption holds, and sexual solenobids are expected to pay the two fold cost of sex. Interestingly, I found that the proportion of sexually reproducing moths correlates positively with the prevalence of hymenopteran parasitoids (II). This result suggests that parasitoids might favour sexual reproduction and help to maintain sexually reproducing moths in locations where more effectively reproducing asexual competitors co-exist. This fits with the "parasite hypothesis of sex" (Hamilton 1980). This assumption would require asexuals to be genetically less variable than the sexuals; then the parasitoids would most likely have more severe effects on the less variable asexuals. Indeed, I found less genetic variation among asexual species than in two sexual species (IV). However, the number of asexual clones was high. Furthermore, I found the most inbred and less variable sexual populations to contain the highest parasitoid prevalence, which suggests that sexual populations with a reduced amount of genetic variation can also be selected against by parasitoids (IV).

In an ecological study (V), I found wood ants to have positive effects on the abundance of fully grown bag worm moth larvae, although the larvae are apparently ideal prey for wood ants. Bag worm moths were most abundant in habitats where wood ants were also abundant and within habitats in trees were wood ants were most numerous. In ant behaviour experiments (V), I found wood ants to aggressively attack parasitoids while bag worm moth larvae remained unharmed. These results suggest an indirect guarding effect of wood ants on bag worm moth larvae. Bag worm moth larvae are probably not easily detected due to their larval case (V). Interestingly, their most harmful enemies, hymenopteran parasitoids, are readily attacked by the ants (V). However, I did not find any evidence that the presence of ants favours asexual *D. fennicella.*

Combined, the costs of sex (II), the additional costs of sex (III) and the observed low levels of heterozygosity due to inbreeding (IV) show that sexual reproduction among solenobid psychids has serious problems. The magnitude of these problems, however, is more than expected by the twofold cost of sex - hypothesis. Asexuality, instead, may be favoured among psychids because many asexual species still remain instead of going extinct. Asexuals may persist partly due to high number of different clones, which was indicated by study IV. What then maintains sexual reproduction among solenobid bag worm moths that bear the costs of sexuality? The co-occurrence of sex and parasitoids in paper II suggests parasitoids to favour sexuality. However, the possibility of some other factors such as reduced parasitoid resistance of asexuals or some unknown abiotic factors can not be excluded. Summarised, the results of this thesis bring rare empirical evidence for the diversity of the factors affecting the occurrence and competitive abilities of closely related sexually and asexually reproducing species. Furthermore, my thesis brings novel information of this poorly studied system.

Acknowledgements

I owe my warmest thanks to my supervisors, Docent Johanna Mappes and Dr. Alessandro Grapputo for their help, guidance and patience along the unexpected and interesting course of my studies. They invested an enormous amount of effort and lots of money to make these studies possible. I also want to thank Professor Rauno Alatalo for his excellent advice concerning the study system, for his supportive attitude towards all the departments´ doctoral students and for his kind assistance with the numerous problems. I want to express my gratitude to Dr. Petri Ahlroth for his ideas and help in planning the study system.

I would like to sincerely thank Kari Kulmala for his invaluable help in fieldwork, laboratory rearing and species determination. Mr. Peter Hättenschwiler and Dr. Erwin Hauser kindly sent me samples from Central Europe and commented my studies in many phases for which I am very grateful. These studies would have been impossible without the excellent previous studies by Mr. Peter Hättenschwiler and the late Professor Esko Suomalainen. I also want to thank Juhani Itämies for his valuable help and advice concerning the study system and species and for commenting on the phylogenetic study. I thank Anna-Mari Koskela and Tapani Koskela for helping to collect samples of *D. lichenella* in the archipelago of Rauma and Reijo Siloaho for his help collecting the samples in Sonkajärvi. I also thank Professor Mats Björklund, Dr. Jukka Jokela, Dr. Pia Mutikainen, Satu Paukku, Mikael Puurtinen and all members of the "round table meeting" for their very helpful comments on the manuscript. Maxine Iversen, Dr. Karolyn Emily Knott and Suzy Mills kindly commented on the language of several papers and the thesis. I thank Gergely Varkonyi for determining the parasitoid species, Jari Kaitila for helping to gather the literature at the beginning of my studies and Jaana Suutari for her help and expertise in gathering the data for the habitat characteristics analysis.

Thanks also to Elisa Kiukkanen for maintaining conditions in the laboratory. I also want to sincerely thank Merja Aho, Panu Halme, Ville Heino, Milja Heikkilä, Satu Helppolainen, Teppo Hiltunen, Katja Huhtala, Riikka Högmander, Antti Kananen, Irma Kananen, Anna-Mari Kolehmainen, Liisa Mattila, Timo Messala, Marjaana Myllylä, Ulla Nivukoski, Susanna Palmu, Tuija Puranen, Kati Sankala, Paula Sarkkinen, Ilja Savolainen, Anja Veijanen, Ilona Yliniemi and many others who helped me in the laborious fieldwork, laboratory rearing and planning the study system and who gave valuable help and guidance along the diverse stages of my studies;. Special thanks go to Dr. Silja Parri for helping to develop the genetic markers and.

Above all, I want to thank Susanna and Ossi for their support and patience in all phases of the work and especially for keeping me motivated. I am grateful to my parents Ritva and Timo, to my sister Milla and to Terttu and Reijo Suonpää for their support and especially for Susanna and my parents for following and encouraging my entomological interests. Special thanks to Mika,

Pekka and Mika, the respected members of the "Tarhamäki Foundation" for their ever lasting positive attitude and for unforgettable adventures that will hopefully continue in future. Thanks to all of my friends, colleagues and all those people that I should have mentioned here.

This study was done at the University of Jyväskylä and it was funded by the Academy of Finland (project numbers 45812, 768945, 779874) under the Centre of Excellence programme.

YHTEENVETO

Lisääntymisstrategioiden evoluutio ja säilyminen pussikehrääjillä (Lepidoptera: Psychidae)

Väitöskirjassani tutkin suvullisen ja suvuttoman lisääntymisen syntyä ja suvullisen lisääntymisen säilymistä *Dahlica* ja *Siederia* sukuihin kuuluvilla pussikehrääjät-heimon perhosilla. Pussikehrääjät on pienehkö, mutta lajistoltaan, ekologialtaan ja evolutiiviselta historialtaan hyvin puutteellisesti tunnettu heimo, ja sen arvellaan käsittävän noin 22 Suomessa esiintyvää lajia. Pussikehrääjät ovat pienikokoisia perhosia. Lajien toukat kantavat mukanaan itse kehräämäänsä, hyvin ympäristöön naamioituvaa toukkapussia, jonka sisään ne voivat vaaran uhatessa piiloutua. Toukat elävät puiden tyvillä ja kivien päällä kasvavilla sammalilla. Yksilönkehitys kestää tavallisesti kaksi vuotta; tästä aikuisvaihe on vain muutaman päivän mittainen. Tutkimuslajini käsittivät seitsemän morfologialtaan ja ekologialtaan hyvin samankaltaista lajia, joista kolme on partenogeneettisiä; lisääntyminen tapahtuu neitseellisesti, ilman koiraita - näistä kolmesta lajista tunnetaankin vain naaraita. Partenogeneesi on harvinaista perhosilla; pussikehrääjillä sitä kuitenkin esiintyy melko yleisesti. Suvullisesti ja suvuttomasti lisääntyvien, hyvin samankaltaisten lajien esiintyminen samoilla alueilla sekä näillä lajeilla yleisesti loisivien pistiäisten esiintyminen paikallisesti hyvin vaihtelevissa kokoonpanoissa loi mielenkiintoiset puitteet tutkia suvullisen ja suvuttoman lisääntymisen suhteellista kehittymistä ja niiden keskinäistä kilpailukykyä.

Ensimmäisessä osatutkimuksessa tutkin sukujen *Dahlica* ja *Siederia* pussikehrääjien sukulaisuussuhteita ja suvuttoman lisääntymisen (partenogeneesi) kehittymistä näillä lajeilla. Tähän tutkimukseen keräsin kaikkien suomalaisten lajien sekä useiden ulkomaisten lähilajien näytteitä. Näiden näytteiden mitokondrio-DNA:n perusteella rakennettiin sukupuu, jonka avulla lajien välisiä polveutumis- ja sukulaisuussuhteita oli mahdollista arvioida. Tässä tutkimuksessa sain selville, että kaikki sukujen *Dahlica* ja *Siederia* lajit ovat toisilleen hyvin läheistä sukua. Joidenkin lajien asema sekä lajien väliset rajat ja lajien määrittäminen pelkästään morfologisten ominaisuuksien perusteella näyttäisivät tutkimuksen perusteella kyseenalaisilta. Ehkä mielenkiintoisin tulos oli kuitenkin se, että tässä lajiryhmässä suvuton lisääntyminen näyttäisi kehittyneen suvullisesti lisääntyvistä kantamuodoista kolme kertaa toisistaan riippumattomasti. Koska useat suvuttomasti lisääntyvät lajit ovat säilyneet nykyhetkeen asti, voitaisiin suvuttomasta lisääntymisestä olettaa olevan pussikehrääjille enemmän etua kuin useimmille muille perhosille, joilla suvuton lisääntyminen on hyvin harvinaista.

Toisessa osatutkimuksessa tutkin suvullisen lisääntymisen säilymisen edellytyksiä pussikehrääjillä. Teorian mukaan suvuton lisääntyminen on suvullista tehokkaampaa, koska siinä vältytään koiraiden tuottamisen kustannuksilta. Tutkimuksessa yleisimmän suvullisesti lisääntyvän lajin *S.*

rupicolella sekä yleisimmän suvuttomasti lisääntyvän lajin *D. fennicella* elinkiertopiirteissä ei havaittu eroja. Tämä tulos tukee suvullisen lisääntymisen (=seksin) kustannusten olemassa oloa. Kun suvullisesti ja suvuttomasti lisääntyvien ja pussikehrääjien osuuksia eri populaatioissa tutkittiin, havaittiin paitsi että osuudet vaihtelevat huomattavasti eri populaatioiden välillä, myös että suuri suvullisen lisääntymisen osuus korreloi positiivisesti korkean loisprevalenssin (loisittujen osuus kaikista yksilöistä) kanssa. Tämä tulos tukee ns. lois-hypoteesia, jonka mukaan loiset saattavat suosia suvullista lisääntymistä, jos suvullisesti lisääntyvä lajia tuottaa vaihtelevampia jälkeläisiä kuin suvuttomasti lisääntyvä laji.

Kolmannessa osatutkimuksessa tutkin suvulliseen lisääntymiseen mahdollisesti liittyviä ylimääräisiä (lisä-) kustannuksia, jotka edelleen saattaisivat heikentää suvullisen lisääntymisen suhteellista kilpailukykyä. Kahdella yleisimmällä suvullisesti lisääntyvällä lajilla, *S. rupicolella* ja *D. charlottae*, havaitsin koiraiden pystyvän hedelmöittämään menestyksekkäästi kaikki jälkeläiset vain ensimmäiseltä naaraalta, jonka kanssa se paritteli. Tämän jälkeisten parittelujen tuloksena naaraat tuottivat selvästi pienempia toukkamääriä kuin koiraan kanssa ensimmäisenä paritelleet naaraat. Tämä havainto yhdessä sen kanssa, että monet populaatiosta olivat naaras-painotteisia, osoittaa selkeästi, että suvullisella lisääntymisellä on myös lisäkustannuksia, koska koiras voi hedelmöittää vain yhden naaraan jälkeläiset ja muut naaraat siten kärsivät koiraiden puutteesta tai näiden hedelmöittämiskapasiteetin rajallisuudesta.

Neljännessä osatutkimuksessa vertasin suvullisesti ja suvuttomasti lisääntyvien pussikehrääjälajien geneettisen vaihtelun määrää käyttäen selluloosa-asetaatti-elektroforeesia. Havaitsin, että molemmat tutkitut suvullisesti lisääntyvät lajit sisälsivät enemmän geneettistä vaihtelua kuin yleisin suvuttomasti lisääntyvä laji. Kuitenkin myös erilaisten kloonien määrä suvuttomasti lisääntyvällä lajilla oli korkea. Tulokset osoittivat että populaatiot ovat toisistaan selkeästi eriytyneitä ja geenivirta niiden välillä on vähäistä.
Suvullisilla lajeilla heterotsygotian määrä oli alhainen, mikä ilmentää sukusiitoksen vaikutuksia pienissä, eristyneissä populaatioissa. Lisäksi havaitsin, että suvullisesti toisen lisääntyvän lajin populaatioissa loisintapaine (loisprevalenssi) oli korkein niissä populaatioissa, joissa geneettisen vaihtelun määrä oli alhaisin tai joissa sisäsiittoisuutta näyttäisi olevan enemmän. Tämä tulos osoittaa, että myös suvullisesti lisääntyvät lajit voivat kärsiä kohonneesta loisintapaineesta, jos geneettisen vaihtelun määrä niissä alenee.

Viidennessä osatutkimuksessa tutkin kekomuurahaisten vaikutuksia pussikehrääjiin ja niiden esiintymiseen. Tässä tutkimuksessa havaitsin, että pussikehrääjät olivat runsaampia populaatioissa, joissa myös kekomuurahaiset olivat runsaimpia. Populaatioiden sisällä vertasin muurahaisten, pussikehrääjien, loisten ja muiden eliöryhmien runsauksia ns. "muurahais-puiden" ja "ei-muurahais-puiden" välillä. Pussikehrääjien havaittiin olevan runsaimpia muurahaispuissa, kun taas useimmat muut niveljalkaiset, mukaan lukien muut perhostoukat, olivat runsaimpia ei-muurahaispuissa. Tutkin myös kokeellisesti muurahaisten reaktioita pussikehrääjätoukkia kohtaan. Näissä kokeissa havaitsin muurahaisten hyökkäävän aggressiivisesti loispistiäisten kimppuun, kun taas pussikehrääjien toukat olivat muurahaisten kannalta vähemmän kiinnostavia ja säilyivät kokeissa vahingoittumattomina, jos niillä oli suojanaan lajeille ominainen toukkapussi. Ilman pussia olleet toukat joutuivat muurahaisten ravinnoksi kuten loispistiäiset tai vertailuryhmänä käytetyt sääsketkin. Tämän kokeen tulokset osoittavat, että kekomuurahaiset eivät pidä toukkapussissa olevaa pussikehrääjän toukkaa kiinnostavana ravintoeläimenä, mutta hyökkäävät aggressiivisesti useimpien muiden hyönteisten, myös pussikehrääjien luontaisten vihollisten kimppuun. Siten muurahaiset voivat epäsuorasti suosia pussikehrääjiä. Muurahaisilla ei kuitenkaan havaittu olevan vaikutuksia suvullisen ja suvuttoman lisääntymisen osuuksiin populaatioissa.

Väitöskirjassani havaitut suvullisen lisääntymisen peruskustannukset (II), suvullisen lisääntymisen ongelmat (III) ja sisäsiittoisuus (IV) ilmentävät suvullisen lisääntymisen kiistattomia ja moninaisia ongelmia. Yhdessä nämä suvullisen lisääntymisen ongelmat ylittävät selkeästi "two-fold cost of sex" -hypoteesin oletuksen näiden kustannusten kaksinkertaisuudesta suvuttomaan lisääntymiseen verrattuna, ja edelleen korostavat niiden tekijöiden merkitystä, jotka ylläpitävät suvullista lisääntymistä *Dahlica* ja *Siederia* -sukujen pussikehrääjillä. Suvuttoman lisääntymisen edullisuutta tutkituilla lajeilla ilmentää nykyhetkeen asti säilyneiden partenogeneettisten lajien suuri määrä. Suvuttomasti lisääntyvien pussikehrääjien säilymistä on saattanut edesauttaa erilaisten kloonien suuri määrä (IV). Suvullisen lisääntymisen suuri osuus alueilla, joilla myös loisprevalenssi on suurin (II), esittää puolestaan, että loiset suosisivat suvullista lisääntymistä. On edelleen mahdollista, että myös muut tekijät, kuten suvuttomasti lisääntyvien pussikehrääjien mahdollisesti alempi vastustuskyky tai jotkin vielä tuntemattomat abioottiset tekijät parantavat suvullisen lisääntymisen suhteellista kannattavuutta. Kokonaisuutena väitöskirjani osatutkimukset antavat uutta tietoa suvullisesti ja suvuttomasti lisääntyvien lajien välisestä kilpailusta sekä näiden lisääntymisstrategioiden kannattavuuteen vaikuttavista tekijöistä. Väitöskirjani antaa uutta tietoa myös aiemmin hyvin puutteellisesti tunnetusta systeemistä.

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ORIGINAL PAPERS

I

PHYLOGENY AND THE EVOLUTION OF PARTHENOGENESIS IN FINNISH BAG WORM MOTH SPECIES (LEPIDOPTERA: PSYCHIDAE) BASED ON MTDNA-MARKERS

by

Tomi Kumpulainen, Alessandro Grapputo & Johanna Mappes

Manuscript (submitted)

https://www.researchgate.net/publication/239802756

https://www.jstor.org/stable/23736156

II

PARASITES AND SEXUAL REPRODUCTION IN PSYCHID MOTHS

by

Tomi Kumpulainen, Alessandro Grapputo and Johanna Mappes

Evolution, in press

http://dx.doi.org/10.1554/03-567

https://www.researchgate.net/publication/8371722

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III

MULTIPLE MATING OF MALES DECREASES FITNESS OF FEMALES: ADDITIONAL COST OF SEX IN PSYCHID MOTHS

by

Tomi Kumpulainen, Johanna Mappes & Alessandro Grapputo

Manuscript

Multiple mating of males decreases fitness of females: additional cost of sex in psychid moths

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ABSTRACT

Theories of sexual selection generally assume that males (or sperm) are practically an unlimited resource for females and therefore all receptive females will be inseminated. Recent papers, however, show that in many mating systems, sperm limitation occurs and thus, the risk of remaining unfertilised can be a serious problem for females. In two bag worm moth species, *Siederia rupicolella* and *Dahlica charlottae* (Lepidoptera: Psychidae), population densities are low and sometimes significantly female biased. Females of these species are wingless, very short lived and mate only once. Moreover, winged males are very poor fliers. Thus, it is unlikely that all bag worm moth females are fertilised. Some males are capable of mating with more than one female. However, multiple mating of males significantly decreased the fertility success of females of both species. Females that copulated with previously mated males had dramatically lower egg hatching success than females that copulated with virgin males. This reduction is most likely due to an insufficient amount of male sperm in subsequent copulations. Bag worm moth females are unable to reject already mated males, and sperm shortage is likely to occur in natural populations. We suggest that our results imply additional costs of sexual reproduction for these species that in nature have to compete with a closely related asexual species in which reproduction is not limited by the availability of males.

INTRODUCTION

Since the classic experiments of Bateman (1948), a general assumption has been that males (or sperm) are practically an unlimited resource for females and that all females will be inseminated. Certainly, the number of mates limits male reproductive success, whereas females are constrained by offspring production, which in turn drives sexual selection for female choice and male-male competition (Bateman 1948; Trivers 1972; Lande 1981; Andersson 1990). Recently, there has been an increasing amount of evidence showing that sperm production is not cost-free and that males have evolved mechanisms for allocating a finite number of sperm to maximise their reproductive success (reviewed in Wedell et al. 2002 B). The observed "cost of sperm" or sperm limitation has seldom been shown to translate into fertilisation success since females are able to compensate for small ejaculations by multiple matings (e.g. Kaitala & Wiklund 1994; Wedell et al. 2002) and sometimes even if the size of ejaculation (spermatophore) decreases with increasing the number of mating, number of sperm can remain the same or increase (Watanabe et al. 1998). In many marine systems, however, sperm limitation is common and various reproductive strategies (e.g. large egg size) have evolved to minimise the female's risk of remaining unfertilised (e.g. Levitan 1998).

Most often the interests of males and females over mating behaviour are dissimilar (see Arnqvist 1989; Arnqvist & Rowe 2002; Chapman et al. 2003). If multiple mating of males causes a decrease in the number of sperm, it will still be beneficial for males to mate with subsequent females and increase the number of their offspring. For females however, mating with an already mated male is detrimental in terms of fewer nutrients from nuptial gifts (see discussion and references in Wedell et al. 2002 A.), increased duration of copulation (Kaitala & Wiklund 1995; Watanabe et al. 1998; Hughes et al. 2000) and increased risk of an unsuccessful fertilisation (Kirkendall 1990). In some mating systems females can compensate for the risks of male multiple mating by choosing virgin males (Wiklund & Forsberg 1985). However, in many mating systems females can not separate unmated males from already mated males (Alatalo et al. 1990) and/or females are incapable of multiple mating (e.g.

Jönsson 1997; Wedell et al. 2002) and thus, the risk of mating with an already mated male can cause females serious fitness costs.

In large populations, a shortage of males or sperm is unlikely to occur particularly in the populations with equal functional sex ratios (see Milner-Gulland et al. 2003), or if females can mate successfully several times (see e.g. Baker et al. 2001). Occasionally, females may have a risk of remaining unfertilised e.g. due to their relatively low mating value (see De Jong & Sabelis 1991) or more rarely due to the occurrence of sterile males (Olsson & Shine 1997). More commonly however, there may be a shortage of potential mates if the functional sex-ratio of the population is adversely distorted (Milner-Gulland et al. 2003). Skewed functional sex ratios and shortage of males may occur in small and sparse populations (as suggested by Puurtinen & Kaitala 2002), where even small fluctuations in sex-ratio result in difficulties for finding mates.

All problems of sexual reproduction not caused by the two-fold cost of sex i.e. the cost of producing males (see Maynard Smith 1978) are called additional costs of sex. Additional costs of sex may emerge in any phase of sexual reproduction (see e.g. Lewis 1983; Arnqvist 1989). Among the most well-known costs is the paradox of female pheromones and other mate attracting signals among insects; active mate attracting is known to cause vulnerability towards parasites and predators (Sahaluk & Belwood 1984; Wing 1988; Vencl et al. 1994; Wertheim et al. 2003). Most studies about the costs of mate calling have concentrated on the costs of male signalling (see e.g. Lloyd & Wing 1983; Cushing 1985; Andersson et al. 1998). Other problems linked with sexual reproduction in general include the increased risk of predation due to resource holding (Sih et al. 1990; Magnhagen 1991), waiting or searching for a mate (Rowe 1994; Acharya 1995; Kotiaho et al. 1998), costs of copulating (Arnqvist 1989; Rowe 1994; Rice 1996, Blanckenhorn et al. 2002), risk of sexually transmitted diseases (Bourtzis & O´Neill 1998), the cellular-mechanical costs of sex (Lewis 1983) and the lack of suitable mates (Puurtinen & Kaitala 2002, Milner-Gulland et al. 2003) or sperm limitation. Furthermore, in species with limited mobility the risk of not finding a suitable mate can be high and has been suggested to drive the evolution of hermaphroditism (Puurtinen & Kaitala 2002) or asexual reproduction (Kumpulainen et al. unpubl.). Additional costs of sex are most likely present in many sexual species, but their importance should be more relevant in natural systems where sexual reproductives have to compete with co-existing asexual reproductives. Additional costs of sex is able to remarkably decrease the average reproductive output of a sexual population (see Lewis 1983; Magnhagen 1991) and the costs of sexual reproduction will be far more than the two fold cost of sex alone (Lewis 1983).

In solenobid psychid moths (genera *Dahlica* and *Siederia,* Lepidoptera: Psychidae), both sexually and asexually reproducing species occur in the same habitat patches (Kumpulainen et al. unpubl.). These closely related species compete for the same resources and share the same parasitoids (Kumpulainen et al. in press). The "all-else-equal" assumption seems to be true for these species; they produce approximately the same amount of offspring. Thus, it is likely that any additional cost of sex for the sexually reproducing species can seriously decrease their competitive ability. We investigated the sex ratios at *S. rupicolella* and *D. charlottae* in 21 and nine different locations of respectively. We also investigated the ability of males to mate multiply and how this translated into female reproductive output. Our aim was to assess if the cost of sexual reproduction could be more than the twofold cost of sex alone for two sexual moth species.

METHODS

Study System

In Finland, seven species of solenobid bag worm moths are known to occur (Suomalainen 1980). In Finland, Solenobinae (Lepidoptera: Psychidae) contains two genera *Siederia* and *Dahlica*, among which the Finnish species are both morphologically and ecologically very uniform. In Central Finland, the most common species are the sexual *S. rupicolella* and the asexual *D. fennicella*. In certain populations, the sexual *D. charlottae* is also among the most common solenobid species.

Bag worm moth larvae carry a larval case (or sack) that is made of fragments of plant material, sand and other dry matter (Suomalainen 1980; Hättenschwiler 1997). Solenobid psychids are relatively small-sized moths; the length of an adult female of the study species is on average 3-5 mm and the wingspan of male on average 13-16 mm. Adults are not able to forage and all nutrients are gathered during their larval development. The life cycle of solenobid bag worm moths from egg to adult takes from one to two years, but the adult stage takes only 3-6 days (Suomalainen 1980). Solenobid larvae feed on moss and algae that grow on the bases of tree trunks, on large tree roots exposed from the ground and on rocks and stones. The larvae climb on tree trunks or rocks for moulting and fully-grown larvae climb to pupate within their larval case. Adult females of *Dahlica* and *Siederia* species are wingless, sessile and incapable of dispersal. Once hatched, sexual females excrete pheromones to attract males. Females of all psychids are known to mate only once (see Kozhanchikov 1956; Hättenschwiler 1997). Shortly after copulation, females lay their eggs in their own larval case and die within few hours (pers. obs.). Males are always winged and fly towards a female guided by female pheromones. Males have a very limited dispersing ability and they can only fly for short distances (between 10 and 100 m).

Sex-ratio Studies

The sex ratios of S. *rupicolella* and D. *charlottae* were studied in April 2000 in 27 populations. However, some populations were too small (less than 10 individuals were found) to perform reliable estimates of their sex ratio. In the final analysis, we included 21 S. *rupicolella* and nine D. *charlottae* populations. The study area is situated in Jyväskylä, Central Finland (62 °15 N', 25°43 E'). Study locations are mainly older forest patches, separated by lakes and fields, or sometimes by human settlements. Forests are dominated by Norwegian spruce (*Picea abies*) and Silver birch (*Betula pendula*), including a few specimens of Scots pine (*Pinus sylvestris*) and occasionally European aspen (*Populus tremula*) and Grey alder (*Alnus incana*). The study populations (Table 1) are situated in Central and southern Finland (see Fig. 1). The detailed habitat description of the locations is given in a study by Kumpulainen et al. (in press).

In order to catch final instar larvae that climb on tree trunks to pupate in early spring, we set tape traps on tree trunks where larvae are immobilised and can later be collected. The traps were set before the beginning of the "climbing period" (30 March to 7 April) and were checked twice per week until the "climbing period" was over (30 April). Each larva was separately labeled, canned and taken into the laboratory for further studies. In the laboratory, all collected larvae were kept individually in transparent plastic containers (height 6.7 cm; diameter 3.7 cm) with foam lids to allow enough oxygen to pass. Temperature and humidity were kept at outdoor levels. The larvae pupated soon after collection and, about three weeks later, males and females began to hatch. The species were determined for all hatched adults (see Kumpulainen, T., Grapputo, A. & Mappes, J. in review). Parasitoid prevalence was high (up to 100 %) in many populations and the sex ratio was determined from the moths that survived from parasitism. The sex ratios of both species were tested assuming equal sex ratio using the X^2 test.

Multiple Mating Experiments

Individuals for multiple mating experiments were collected in April 1999 and April 2000. In the latter year they were collected simultaneously with the study individuals for sex ratio studies. After hatching from pupa, females climbed on the top of their own larval case and started releasing pheromones and waiting for hatched males to arrive. At this point, we offered a randomly chosen male to each female and let them copulate. Copulation normally succeeded immediately and lasted between 5 and 30 minutes. After copulation, females laid their eggs inside their own larval case. Females never waited for another copula or for another male and died within a few hours after egg laying. Thus, it is clear that they copulate and reproduce only once. If the male was still able to fly after the first copula (mostly 0-24 hours after the first copula), it was offered a chance to copulate with a second, third, fourth and fifth female and these copulations occurred as described above. In 1999, we mated 53 S. *rupicolella* and 69 D. *charlottae* among which only 8 and 40 were able to multiple mate, respectively. In 2000, we mated 111 *S. rupicolella* and 69 *D. charlottae* among which 47 and 46 males carried out multiple matings, respectively. The time between matings varied from 4 minutes to 69 hours in *S. rupicolella* and from 5 minutes to 48hours in *D. charlottae* . Egg clutches laid by individual females were kept separate. The number of hatched larvae was calculated for each female and the numbers of larvae per female were compared among females that were mated with the same males.

RESULTS

Sex Ratio

In *S. rupicolella,* we found a significant female bias in 4 out of 20 studied populations. The proportion of males in those populations varied from 8% to 41%. One population was significantly male biased (56% of males) whereas in 15 populations sex ratio did not differ significantly from expected 50:50 ratio (Table 1). Thus, we can argue that occasionally, the availability of males may limit the fertilisation success of females in this species.

In *D. charlottae,* one population out of 9 was significantly female biased (the proportion of males was 21%), and 5 populations were significantly male biased. Three populations out of nine did not differ significantly from expected 50:50 ratio (Table 1).

Multiple Mating and Male Fertilisation Success

For both species, neither the origin of females nor the origin of males affected the number of hatched larvae when a female was mated with a virgin male, in either study year (all p values are > .134). Thus, the origin of a female and a male was excluded from future analyses. The number of hatched larvae decreased with increasing number of copulations a male had achieved in both species (Figs 2 and 3). When mated with once previously mated males *S. rupicolella* females produced 59% and 29% less larvae than females that mated with virgin males (paired t-test: t = 2.55, df=7, p = 0.038; t = 3.38, df=46, p = 0.001) in 1999 and 2000, respectively. In the same two years, when mated with males that had already mated twice, the females produced, 100% and 44% less larvae than the females mated with the virgin males (t=3.9, df=2, p = 0.060; t=3.2, df=29, p = 0.001). *D. charlottae* females suffered a 24% and 24 % loss in the number of produced larvae when mated with once previously mated males (t=2.26, df=39, p = 0.029; t=2.35, df=45, p = 0.023) in 1999 and 2000, respectively. When mated with males that had already mated twice, females suffered a 29% and 44% reduction in the number of produced larvae (t=2.54, df=9, p = 0.032, t=2.43, df=14, p = 0.029) in 1999 and 2000, respectively. Time between the first and the second mating did not affect the number of hatched larvae in *S. rupicolella* (rs = -.14, p = .390, n = 43). In *D. charlottae* males that were allowed to recover longer between matings sired less offspring compared to males that mated soon after the first mating (rs = -.35, p = .021, n = 43). These results suggest that short recovery time between matings do not explain the decline in offspring number. Thus, it is obvious that mating with previously mated males causes a serious reproductive cost for psychid moth female.

DISCUSSION

We found that males of *S. rupicolella* and *D. charlottae* were unable to fertilise the progeny of the second and third females as successfully as the first female (see figs. 2 and 3). Multiple mating by males caused a significant drop in the proportion of hatched eggs in both psychid species causing significant fitness costs for the females. Multiple mating by males has previously been shown to lead to a reduction in spermatophore size i.e. the amount of nutrients a male provides females during copulation, in several butterfly species (Watanabe et al. 1998; Rutowski et al 1987). Often, however, diurnal butterfly females are able to compensate for this reduction with multiple matings. Moreover, they can actively choose unmated males as their mating partners (e.g. Wiklund & Forsberg 1985). Psychid moth females are not known to obtain spermatophores or other energy rich resources from the males to improve their survival or reproductive output. Thus, the reduction in the reproductive output of females was due to a shortage of sperm, not nutrients. For psychid moth females, multiple mating by males caused a significant fitness cost because females are not able to mate multiple times. Nor do they have any mechanism for active choice of fertile males (Hättenschwiler 1997). Moreover, in small, female-biased populations too few males would be available for any female choice even if the sex ratio is close to 50:50. This is due to a potentially biased functional sex ratio, if females and males do not hatch simultaneously. Moreover, male psychid moths are very poor fliers and females are entirely wingless thus eliminating the possibility for active mate search. Therefore, functional sex ratio may be seriously female-biased particularly in small and sparse populations, which are typical for these species. Thus it is likely that, in nature, many females fail to lay any fertilised eggs due to lack of males and sperm.

In our mating experiment, males were allowed to mate with subsequent females if they were able to fly after their first mating. Some insects are known to transfer more sperm after a longer recovery period (see e.g. Arnqvist & Danielsson 1999). In this study, short recovery time does not explain the reduced fertilisation success of females since males with longer recovery period (before the second mating) sired less offspring than males that mated soon after the first mating. Moreover, in nature, psychid moth males are unlikely to have a long recovery period, because their time and space is limited by the presence of their natural enemies (such as wood ants and insectivorous birds), by their very short adult phase and by their poor dispersal abilities (see Hättenschwiler 1997). Subsequently, adult males are unlikely to survive till the next day and their chances of multiple matings are restricted to their first day of adulthood. Thus we suggest that our experiment does not overestimate the cost of mating with an already mated male.

In the present study, most males (approximately two thirds of all males) were unable to copulate more than once. Moreover, approximately 50% of the males were not even able to fly anymore after the first mating. Poor longevity of males is surprising since selection should strongly favour males that can successfully mate multiply. One possibility is that our study populations are somehow unhealthy, which would reflect the problems in reproduction. Recently we found that populations of *S. rupicolella* and *D. charlottae* are seriously inbred (Grapputo et al. unpubl.) which may be one possible explanation for the reduction in male longevity. Inbreeding often reduces fertilisation success (Charlesworth & Charlesworth 1987; Jiménez et al. 1994; Keller & Waller 2002) and recently it has been suggested that male sexual traits can be_particularly sensitive to the effect of inbreeding due to their condition dependent nature (Møller 2000; Ahtiainen 2003).

In our study system sexually reproducing moths (*S. rupicolella* and *D. charlottae*) often co-occur and compete with *D. fennicella*, a strictly parthenogenetic species. *D. fennicella* produce only females (Suomalainen 1980) and is thus able to avoid the cost of producing males (see Hamilton 1980) and also avoid all additional costs of sexual reproduction. Parthenogenetic *D. fennicella* should outcompete sexual *S. rupicolella* and *D. charlottae* since they clearly suffer more than only the two-fold cost of sexual reproduction. In a previous study, we found that sexual *S. rupicolella* is more common in habitats where hymenopteran parasitoids are common. Thus it is possible that high parasitoid loads maintain sexual reproduction in this system, while asexual reproduction has a selective advantage in parasitoid free habitats. In solenobids, parthenogenesis has evolved independently several times (Seiler 1961, 1963, Lokki et al. 1975; also Kumpulainen, T., Grapputo, A. & Mappes, J. unpublished) while it is very rare among other Lepidoptera. Thus, it is possible that low mobility, together with poor fertilisation success, has selected for parthenogenesis in this group.

Acknowledgements

We want to acknowledge K. Kulmala for his assistance in field collecting and also in laboratory studies. We also want to thank P. Halme, V. Heino, T. Hiltunen, I. Kananen, A.-M. Kolehmainen and L. Mattila for their assistance in the field and laboratory work and Suzie Mills, Esa Koskela, Mats Björklund and

Pia Mutikainen for their helpful comments on the manuscript. Esa Koskela helped with statistics. This study was conducted at the University of Jyväskylä and it was financially supported by the Academy of Finland, project number 779874.

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TABLE 1 Sex ratios of *S. rupicolella* and *D. charlottae* in 20 different populations. Numbers show the amount of females and of adults in total. Female biased sex ratios are shown in bold type with star.

S. rupicolella	**Hj**	**Hn**	**Isa**	**Kv**	**Kö1**	**Lv1**	**Lv2**	**Lv3**	**Lv4**	**Lv5**	**Mu**	**Pen**	**Pih**	**Sala**	**Sip 1**	**Sip 2**	**Sip 3**	**Sj**	**Vilj**	**Vill**
Proportion of females	0.48	0.73	**0.92** *	**0.86** *	0.44	0.60	0.60	**0.59** *	0.56	0.37	**0.70** *	0.38	0.47	0.56	0.59	0.51	0.42	0.38	0.56	0.62
Total number of males + females	42	11	12	14	339	45	48	165	32	38	33	32	58	43	70	107	12	39	54	34
D.charlottae																				
Proportion of females	0.44				0.22	0.27	0.46	0.33			**0.79** *		0.45	0.36				0.25		
Total number of males + females	32				165	30	37	67			14		29	14				40		

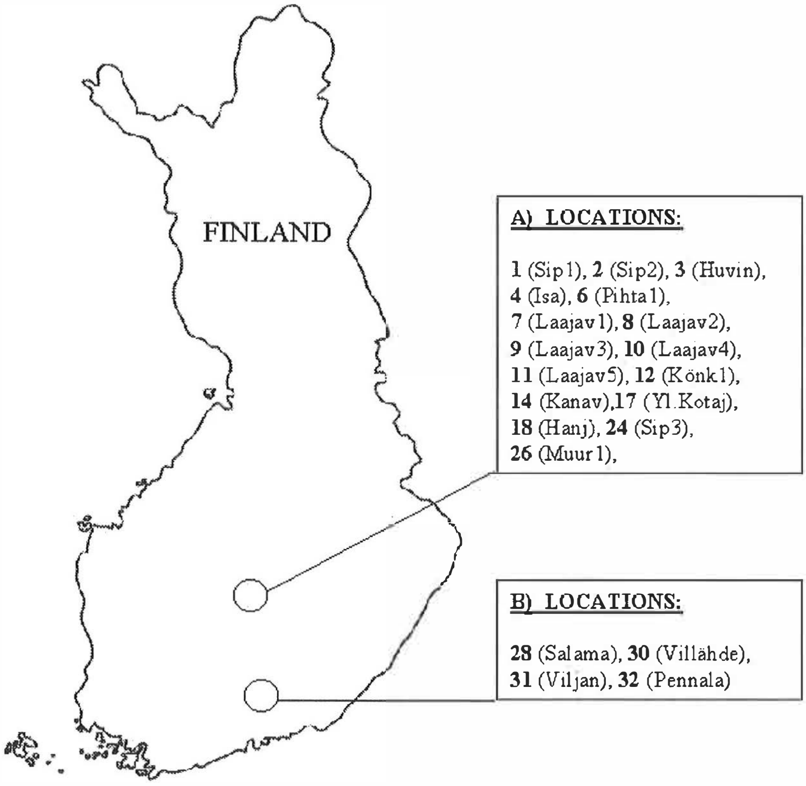

FIGURE 1 Map of Finland. The study areas are indicated with spots. A) The main study area in Central Finland, locations 1 - 26, B) Area of the southern populations, locations 28 - 32.

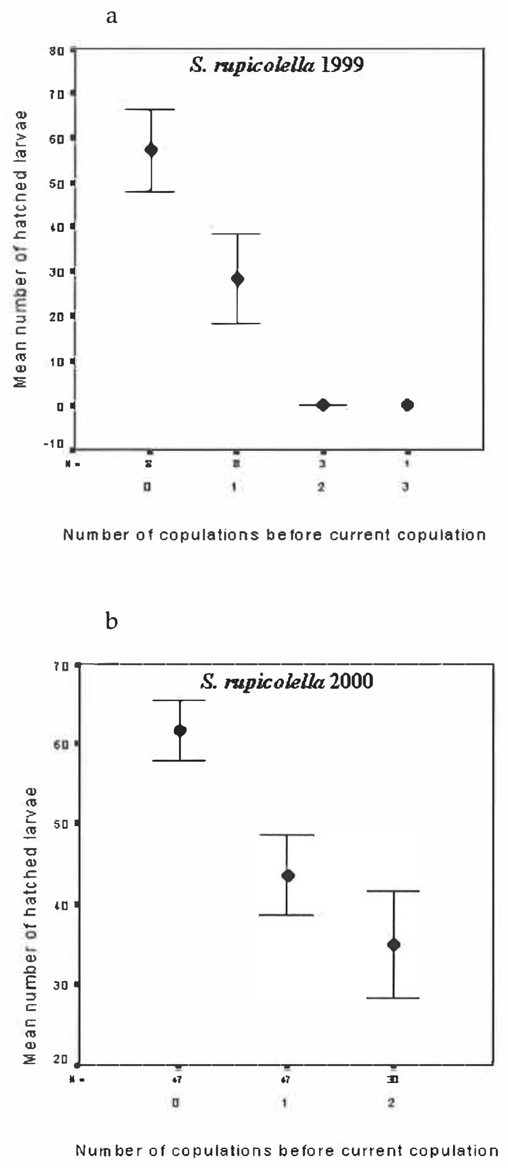

FIGURE 2 Mean number of hatched larvae from each copulation of male *Siederia rupicolella* (a) in 1999 and (b) in 2000. X-axis indicates the number of copulations for males previous to the current copulation.

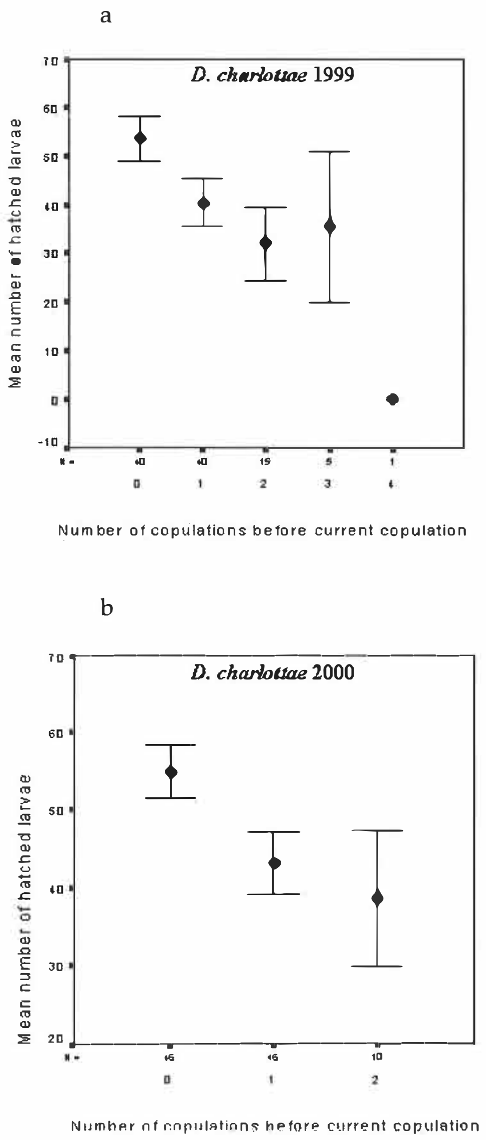

FIGURE 3 Mean number of hatched larvae from each copulation of male *Dahlica charlottae* (a) in 1999 and (b) in 2000. X-axis indicates the number of copulations for males previous to the current copulation.

IV

GENETIC DIVERSITY AND PARASITOID PREVALENCE IN POPULATIONS OF ASEXUAL AND SEXUAL BAG WORM MOTHS (LEPIDOPTERA: PSYCHIDAE)

by

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Manuscript (submitted)

https://doi.org/10.1186/1472-6785-5-5

V

ANTS HAVE POSITIVE EFFECTS ON THE ABUNDANCE OF UNTENDED BAG WORM MOTHS (LEPIDOPTERA: PSYCHIDAE)

by

Tomi Kumpulainen, Johanna Mappes & Alessandro Grapputo

Manuscript

Ants have positive effects on the abundance of untended bag worm moths (Lepidoptera: Psychidae)

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ABSTRACT

Many ant species are general predators that hunt and feed on a wide range of prey species. Ants are known to reduce the amounts of some arthropods, such as most Lepidoptera larvae. Less well known, however, is that ants also cause indirect benefits to other arthropods by reducing the amount of their enemies. We found that untended bag worm moth larvae (Lepidoptera: Psychidae) were abundant in forest habitats where wood ants (*Formica rufa* group) were also abundant. Within habitats, trees patrolled by ants support significantly more psychid moth larvae and significantly less larvae of other Lepidoptera. Parasitoids and predatory insects were also less abundant on these trees. We monitored ant reactions towards psychid larvae and their enemies in three different behavioural experiments and found that ants aggressively attacked parasitoids while psychid larvae, within their larval cases, remained unharmed. Larvae without the larval case were readily attacked by ants. We suggest that the case protects bag worm moth larvae from ant predation. Most likely ants do not recognize psychid moth larvae as prey items when in the larval case. While ants do not tend psychid moth larvae, they indirectly promote the moths' survival by predating their natural enemies such as spiders and parasitoids.

INTRODUCTION

Wood ants of the Northern Hemisphere (*Formica rufa* group) are aggressive predators (see e.g. Collingwood, 1979; Skinner & Whittaker 1981, Hölldobler & Wilson 1990, Halaj et al. 1997) that hunt on tree trunks and forage on spiders and other invertebrates (e.g. Fowler & MacGarvin 1985; Haemig, 1994, Aho et al. 1997; 1999). Common in the coniferous forests of Northern Europe, wood ants are known to alter the distribution and abundance of many arthropods such as Lepidoptera larvae (Laine & Niemelä, 1980; Skinner & Whittaker, 1981; Karhu, 1998) and spiders (Laine & Niemelä 1980; Haemig, 1994; Aho et al. 1997). As effective predators, ants are also known to compete for food resources with numerous (including very distant) taxa (Hochberg & Lawton, 1990; Haemig, 1994; Halaj et al. 1997; Aho et al. 1997, 1999). They are undoubtedly a key component of northern coniferous forest ecosystems (see e.g. Collingwood, 1979; Fowler & MacGarvin, 1985; Hölldobler & Wilson 1990).

Most studies of ant predation have focused on the direct effects of ants on other animal species. Importantly, ant attendance is known to be significant for many tended insect species because ants remarkably reduce the risk of being parasitized or predated by other arthropods (Pierce & Mead, 1981; Bristow, 1984; Völkl, 1992). Ants are known to favour and tend aphids (Skinner & Whittaker 1981; Fowler & MacGarvin, 1985; Karhu, 1998; Völkl et al., 1998; Flatt & Weisser, 2000) and certain butterflies (e.g. Thomas & Elmes, 1993; Weeks, 2003), earth worms (Laakso & Setälä, 1997) and beetles (Päivinen et al. 2002). Many studies have tried to resolve whether ant effects on the forest ecosystem are positive or negative (e.g. Aho et al. 1999; Sipura, 2002; Lenoir et al. 2003). Most studies have reported negative or no effects of ants on untended arthropods (e.g. Faeth, 1980; Aho et al. 1997 and 1999; Halaj et al. 1997). However, ants also have indirect positive effects by providing an effective shelter for arthropods that have evolved defences against ant predation.

Psychid bag worm moths (Lepidoptera: Psychidae) are not tended by ants. On the contrary, they can be regarded as potential ant prey, along with most other

Lepidoptera larvae. Psychids are small insects with a life cycle lasting from one to two years. The adult stage takes only 3-6 days (Suomalainen, 1980), however, while the larval phase is very long (one to two years). Psychid larvae are patchily distributed but usually abundant within their locations. They are not known to posses effective anti-predatory features such as spines, hairs, hard exoskeleton, good escaping abilities, poison or unpalatability (see e.g. Hättenschwiler, 1997). Still we have observed that psychid moths live in the immediate vicinity of ant nests, feeding on the bases and trunks of Norwegian spruce (*Picea abies*) with intense ant traffic.

Bag worm moth larvae (genera *Dahlica* and *Siederia*) feed on the moss and algae growing at the base of trees, on large, exposed tree roots, and on rocks and stones. The larvae climb on tree trunks or rocks for molting and fully-grown larvae climb trees with their larval case to pupate within it. Bag worm moth larvae carry a larval case (or sack) made of fragments of plant material, sand and other dry matter, throughout their larval development. The larval case covers most of their body, leaving just head and legs visible when the larva is moving. When disturbed, the entire larva can hide inside its case.

We investigated if wood ants (*Formica rufa* group) recognise or consider psychid moth larvae and their parasitoids suitable prey and if ants affect the numbers and distribution of psychid moths either by predating psychid larvae or by reducing the number of their enemies. Psychid moths' natural enemies include common lethal hymenopteran parasitoids, by which larvae are heavily infected and predatory insects, such as spiders and many Coleoptera and Hemiptera species. Coleoptera (adults and larvae) usually prey on the fully-grown larvae (Kumpulainen & Kulmala, personal observations, 1999-2003). If ants do not hunt psychid moths, while they predate on the parasitoids that infect psychid moths, they might even favour asexual reproduction of the psychids. This would be due to reduced parasitism by the hymenopterans, which are expected to favour sexual reproduction among their hosts (see Kumpulainen et al. in press). In this study, we measured both direct and indirect effects of wood ants on psychid moths and on hymenopteran parasitoids by: 1. assessing which environmental factors, including ant numbers, affect the local abundance of bag worm moths; 2. comparing moth, parasitoid and general arthropod numbers on trees patrolled by ants (ant trees) and on trees with little ant traffic (non-ant trees); and 3. testing if moth larvae are considered prey by ants.

MATERIALS AND METHODS

Study species

Finland is home to seven species of solenobid (genera *Dahlica* and *Siederia*) bag worm moths (Lepidoptera: Psychidae) (Suomalainen 1980). These solenobid species are both morphologically and ecologically similar. *S. rupicolella* (sexual) and *D. fennicella* (asexual) were chosen as our study species because they are the most common species in the study area (80% or more of all species of Psychidae moths in Central Finland) and are often associated with wood ants.

Adult psychid moths have limited dispersal ability with females being incapable of dispersal and males being able to fly for short distances only (between 10 and 100 m). Once hatched, sexual females excrete pheromones to attract males. After copulation, females lay their eggs in their own larval case and die within a few hours of egg laying. Parthenogenetic females, instead, do not excrete pheromones but start to lay eggs shortly after hatching from pupa and then die. The length of an adult female is, on average, 3-5 mm and the wingspan of the male averages 13-16 mm. Larvae size is equal to the length of an adult female and the larval case averages 6-8.5 mm (see Suomalainen 1980, Hättenschwiler 1997).

Natural enemies of psychid moths

Probably the most harmful enemies of solenobid larvae are hymenopteran parasitoids. In some moth populations, up to 100% of larvae are infected (Kumpulainen et al. in press). At least two common species of Hymenopteran parasitoids (*Orthizema* spp.: Hymenoptera: Ichneumonidae) infect solenobid larvae (see Hättenschwiler, 1997; also Hättenschwiler personal comm.), and probably a few other, rare species also. These hymenopteran parasitoids are similar in length to wood ant workers, 7-9, mm but are more slender. Parasitoid females normally lay one egg per host larva and the parasitoid eggs remain dormant until the fully-grown host larvae pupate. The parasitoid larva then quickly eats the host, pupates and hatches.

Study area

Within an area of 16 x 8 km close to the city of Jyväskylä, Central Finland, we chose 38 study sites (average size 1 030 m^2) representing typical psychid moth habitats. Sites consisted of the warmest edges of older forests and areas of sparse forest.

Habitat characteristics

To investigate which habitat characteristics have an effect on bag worm moth abundance, we measured habitat size, forest age, main tree species, coverage (in terms of percentage) of the main vegetation layers (mosses, ground layer, tree top), temperature variation, vascular plant and moss species diversity, lichen abundance and ant abundance in 38 study locations. All habitat and vegetation investigations were performed by the same investigators (J. Suutari & T. Kumpulainen) in August 2001. We set a max-min thermometer in each location to measure maximum and minimum temperatures. The thermometers were installed at the beginning of August 2001 and removed at the end of August 2002.They were placed at the base of trees (minimum diameter 30 cm at the height of one meter), on the shadow side of the tree trunks. All thermometers were situated within meters of the forest edge, where psychid moths were most abundant. The thermometers were checked for the highest and lowest recorded temperatures at the end of each month. Average temperatures and standard errors of average temperatures were determined for all study locations for the entire study period (13 months).

In each study location, we counted the number of wood ant nests (Hymenoptera: Formicidae: *Formica rufa* group) and ant paths. Similarly, we counted ant traffic on five randomly selected spruce trunks (*Picea abies*) or, if no spruce were available, on birch (*Betula pendula)* of equal size (diameter 30-40 cm) in each location. Ant traffic was measured in sunny weather, approximately + 20 °C (+/- 2 °C). Before counting ants, we set an imaginary line at the height of one meter and then counted all wood ants crossing this line upward during a period of three minutes. This procedure was performed twice for all studied trees and the average was calculated. The average ant traffic for each locality was calculated on the basis of these five trees per location. We determined an additional ant activity value (between 1-5) for all locations by assessing the average ant activity at the edge areas of the forest patches.

Principal component analyses were performed separately for ant associated and forest descriptive variables.

Ant trees versus non-ant trees

Because locations differ in many characteristics, correlative comparisons can give confounding results. Thus, we studied the direct effects of ant predation on psychid moths, their natural enemies and other relevant arthropod taxa within locations. We set tape traps on the trunks of 36 pairs of trees (Norwegian spruce, *Picea abies*) in six study sites in which psychid moths and *F. rufa* colonies were abundant. Within a pair of trees, there was always a tree with heavy ant traffic and another tree without or with only minor ant traffic (1-5 ants at a time). The diameter of the trunks (25 cm minimum) and the distance of the trees from edge of the forest was the same within each pair. This allowed for direct comparison of ant traffic. The traps were set in the beginning of August 2001 and were checked twice every month until the beginning of October 2001. The traps were reset in April 2002 and checked until the end of October 2002. Tape traps collected most of the arthropods that climbed (or rested on) the tree trunks, excluding the ants and certain other hymenopterans that are able to walk over tape traps without getting stuck. At each check, we identified, counted and removed all arthropods we found and counted the number of ants on each of the study trees. The collected arthropods were classified into the following groups: small psychid larvae, large psychid larvae, ant prey animal (including non-psychid Lepidoptera larvae, adult Diptera, Orthoptera, chrysomelid beetles, Trichoptera, Hymenoptera except ants, and parasitoids that infect psychid moths) and predatory arthropods (spiders, harvest-spiders and predatory Coleoptera; e.g. carabids and staphylinids). The number of specimens for each group was tallied from the whole collecting period. These total numbers were compared between ant trees and non-ant trees using repeated measure ANOVA. The number of different groups of arthropods in ant trees versus non-ant trees was used as the dependent factor and the location was used as the independent factor. The number of parasitoids between ant trees and non-ant trees was compared using Wilcoxon´s test since total number of parasitoids was too low to conduct full ANOVA model.

The reactions of ants towards moth larvae I

We tested the direct reactions of ants towards psychid moth larvae. In three separate behavioural experiments, we facilitated encounters between ants and potential prey items on ant paths (on the ground). In the first behavioural experiment, in June 2001, we studied the aggressiveness of the reactions of wood ants to psychid moth larvae in four separate wood ant colonies in a total of 27 repeats. We attached a strip of sticky brown tape to a wooden board. The board was placed on an ant path and ants were allowed to acclimate to the empty board. We then placed a final instar larva of either *D. fennicella* (n=14) or *S. rupicolella* (n=13), a dry spruce needle (*Picea abies*) (collected from the forest floor) and a freshly killed mosquito on the tape. Prey items were placed 8 cm apart. All prey items were approximately equal in size.

Mosquito is known to be a desirable prey item for ants; spruce needle a less desirable item and thus we had a chance to compare ant reactions towards prey items of varying attraction and moths. The experiments were performed less than two meters from a large spruce (*Picea abies*) and more than five meters from the closest ant nest. Thus, we were able to measure the effects of natural ant traffic, instead of the reactions of ants defending their nest. The reactions of ants towards the prey items were observed during a period of five minutes, or until one of the prey items was taken by the ants. New prey items were used for each separate study period, and the placement of the items on the board was varied. During each study period, we usually observed ants interacting with all prey items offered. The ants' reactions were classified into five degrees of aggression: 0 = no reaction to encountered item, 1 = ant stopped at the encountered item, 2 = ant briefly tried to lift the prey item, 3 = ant spent more than one second pulling the prey item in order to detach it from the tape, 4 = ant took the prey item. Each repeat includes 12 to 40 independent behavioural observations. After the experiment, we calculated the mean degrees of ant aggression (per each repeat) towards different prey items and these values were compared between the prey items.

The reactions of ants towards moth larvae II

In a second behavioural experiment, we studied the reactions of ants towards *D. fennicella* (n=10) and *S. rupicolella* (n=14) larvae with and without larval cases. This experiment was performed in four separate colonies of *F. rufa* (12 repeats in total) in August 2001. For half of the larvae the larval case was removed without harming the larvae. Each pair of larvae always consisted of larvae of the same species (one larva with and the other without larval case). The experimental procedures were otherwise similar to the first ant behavioural experiment.

The reactions of ants towards moth larvae III

In the third behavioural experiment, we studied the ants' reactions towards hymenopteran parasitoids (*Orthizema* spp.) and compared them to the ants' reactions towards the larvae of *D. fennicella* (n=15) and *S. rupicolella* (n=3) within their case. This experiment was performed in three different *F. rufa* colonies (18 repeats in total) in July 2002. To make sure parasitoids could not escape, they were killed by freezing (-20 °C) before the experiment (living parasitoids occasionally were able to detach themselves from the tape if they had not contacted the tape with their abdomen, antenna, head and thorax). The experimental procedures were otherwise similar to the previous experiments.

RESULTS

Habitat characteristics

In the comparison between locations, a principal component analysis for all ant variables (number of ant trees, number of ant paths, average ant traffic and activity) revealed one major component indicating ant abundance and activity (Table 1a). The "ant component" correlated positively with moth population density (Figure 1a), controlled by parasite prevalence (Partial correlation $r = 0.63$, $n = 29$, $p < 0.001$). There was no correlation between the ant component and the proportion of sexual reproduction among moth populations (Figure 1 c) ($r_s = 0.114$, $n = 30$, $p = 0.547$). Results clearly show that moth abundance is positively correlated with ant abundance but ants do not influence relative abundance of sexual and asexual species.

Principal component analysis for environmental variables extracted two major components (see Table 1b). The third component was excluded from further analysis because it explained only 14.2 % of the total variation in original variables and it correlated with original variables and did not contain any biologically meaningful information. Neither the first (spruce component) nor the second component (mixed forest component) correlated with moth population density (spruce forest component: $r_s = 0.41$, $n = 38$, $p = 0.809$; mixed forest component: $r_s = 1.56$, $n = 38$, $p = 0.349$). The spruce forest component correlated positively both with the proportion of sexual *S. rupicolella* (Spearman´s correlation $r_s = 0.475$, $n = 30$, $p = 0.008$) and with parasitoid prevalence ($r_s = 0.478$, $n = 32$, $p = 0.006$). The mixed forest component also correlated positively with parasitoid prevalence ($r_s = 0.367$, $n = 32$, $p = 0.039$). There was no correlation between the mixed forest component and the proportion of sexual reproduction ($r_s = -0.04$, $n = 30$, $p = 0.832$). This indicates that sexual *S. rupicolella* is relatively more abundant in older forests but parasitoids are not associated with any particular forest type.

Ant trees versus non-ant trees

The comparison between ant trees and non-ant trees is summarised in Table 2. Larger psychid larvae were significantly more abundant on ant trees than on non-ant trees (see Fig. 2a). The number of aphids was also significantly higher on ant trees. In contrast, the number of all other arthropods, considered potential prey for ants, was significantly higher on non-ant trees than on ant trees, including the numbers of Lepidoptera larvae (apart from psychids). The number of parasitoids tended to be higher in non-ant trees but, due to the low total number of parasitoids, we found the difference was not statistically significant (see Fig. 2b).

The reactions of ants towards moth larvae I

Ants reacted similarly towards the larvae of sexual *S. rupicolella* and asexual *D. fennicella* moths in all behavioural experiments (experiments I to III) (all p values were > 0.140), and thus the data from these two species was pooled for future tests. Simultaneously offered prey types make the aggressiveness scores dependent on each other and thus we used repeated measures ANOVA, which takes into account the dependent structure of the data. In the model, we used mean values of aggressiveness scores for different prey types as the dependent factor. The ant nest (location) was used as the independent variable. No interactions between the ant nest and aggressiveness against the different prey types were found ($F_{(8,44)} = 0.91$, $p = 0.515$). However, different colonies differed significantly in their level of aggressiveness (($F_{(8,44)} = 3.15$, $p = 0.034$). Ant reactions toward different prey types were significantly different ($F_{(2,21)} = 97.2$, $p < 0.001$): ants were significantly more aggressive toward mosquitoes than toward moths but reactions toward moths did not differ from reactions toward needles (within subject contrasts: $F_{(1,22)} = 178{,}6$, $p < 0.001$; $F_{(1,22)} = 3{,}46$, $p < 0.076$, respectively). In all 27 repeats, ants either tried to detach the mosquito from the tape or succeeded in doing so (degrees of aggression 3-4), whereas ants attempted to detach only one larva (one attempt with no success) and none of the needles (Fig 3a.).

The reactions of ants towards moth larvae II

Ants reacted significantly differently towards solenobid larvae with and without their larval case. Ants were found to attack moth larvae more aggressively if the larval case had been removed (Repeated measure ANOVA: $F_{(1,8)} = 74.4$, $p < 0.001$) (see Fig. 3b). In all except one repeat, ants attacked and tried to detach larvae without their case. In seven of 12 repeats, the larva was successfully detached from the tape and taken to the ant nest. Ants tried to detach (with no success) only two larvae within their case. Again, ant colonies differed significantly in their aggressiveness $F_{(3,8)} = 4.70$, $p = 0.036$) but they attacked similarly against different types of prey. This is indicated by the lack of statistical significance in the interactions term ($F_{(3,8)} = 0.96$ $p =0.459$).

Ant reactions towards moth larvae III

We observed significant differences in ant reactions towards solenobid larvae within larval cases compared to reactions towards the hymenopteran parasitoids. When ants encountered moth larvae and parasitoids, ants were found to attack parasitoids more aggressively than they attacked moth larvae ($F_{(1,15)} = 82{,}92$, $p < 0.001$) (Fig. 3c). Ants attacked the parasitoid in all 18 repeats and, in 12 of these repeats, the parasitoid was successfully removed from the tape. All moth larvae remained on the board for the entire 5 minutes and only one of 12 larvae was attacked by the ants in an attempt to remove it from the tape. We did not find any statistical differences between the reactions of ant colonies, nor did we find any interactions between colonies to different prey types ($F_{(2,15)} = 0.53$, $p = 0.599$), $F_{(2,8)} = 0.11$, $p = 0.894$).

DISCUSSION

Results of this study indicate ants' ability to indirectly favour psychid moths, a taxonomic group that has not previously been associated with ants in any way. The two locally most abundant psychid moth species, *Dahlica fennicella* and *Siederia rupicolella*, coexist in the same forest edge habitats with colonies of wood ants (*Formica rufa* group). The density of Psychidae moths correlated positively with ant density (Fig.1.). Moreover, within study locations, psychid larvae, particularly the fully-grown larvae, were more abundant in trees frequently patrolled by ants (Figure 2a, Table 2). This is surprising because bag worm moth larvae appear to be an ideal prey item for wood ants: they are abundant in the area, they are easy to catch, they have no hard exoskeleton or chemical defences and they occur in habitats where ants already patrol. An average sized ant colony has been estimated to gather more than 20 000 moth and sawfly larvae during one day and Lepidoptera larvae often constitute one of the most important food sources for wood ant colonies (Hölldobler & Wilson, 1990). In accordance with previous findings (Skinner & Whittaker, 1981 and Weseloh, 1993 and 1994), we observed that Lepidoptera other than psychid moths were significantly more numerous in trees were ants were not patrolling (see Table 2).

Although psychid larvae are not hunted by wood ants, this cannot be due to unpalatability or other chemical protection because, in the second ant behavioural experiment (Fig. 3b.), 11 out of 12 larvae without a larval case were attacked and all ants behaved very aggressively towards the larvae. Instead, only one psychid larva within its case was investigated by ants and all 12 larvae within their larval case remained unharmed by the ants. Ant reactions towards psychid larvae within their cases were similar to ant reactions towards spruce needles, which clearly were not considered items of interest.

Defences of other Lepidoptera larvae include mimicry, morphological, behavioural and chemical defences, camouflage and myrmecophilous relationships (e.g. Heads & Lawton, 1985; Ito & Higashi, 1991; Codella & Raffa, 1995; Machado & Freitas, 2001). Psychid moth larvae are not known to have any effective predator avoidance strategies or other protection against ants than their larval case. The case however, is usually well-camouflaged and made of sand, forest litter and dead plant matter bound together with a silky thread (see Hättenschwiler, 1997). Such structure and coloration of the larval case probably helps larvae to avoid bird predation. However, it was not previously known how effectively such a larval case could aid psychid larvae to avoid predation by ants. While ants collect large amounts of organic matter used as nest building material (see e.g. Hölldobler & Wilson), they do not seem to collect bag worm moth larvae in big numbers. It is not known, what makes the larval case undetectable for ants. We suggest that ants may be confused by the cover material of case rather than smelling filter because smelling filter does not seem to work against other predatory insects or against parasitoids. The larval case is covered by small particles of sand, forest litter and plant material that is generally of low interest to wood ants. Ants may regard larval case as parts of forest litter instead of potential prey animals.

The abundance of predatory arthropods (spiders and predatory Coleoptera) tended to be lower in ant-trees than in non-ant trees. One possibility for the lack of significance can be due to the presence of spiders that actively hunt wood ants and are thus more likely to occur in ant trees than in non-ant trees. We observed several smallish (3-4 mm) net-building spiders preying on wood ants. Previously, Aho et al. (1997) found that ants reduce the numbers of other arthropods, especially spiders, in spruce dominated forests in Central Finland. Haemig (1994) also offers evidence of ants reducing the amount of insects and spiders in a study of Swedish spruce forests. Since spiders, predatory bugs and several coleopterans prey upon Psychidae larvae (personal observation), it is likely that trees occupied by ants offer a safer habitat for moths which explains, in part, their higher incidence in ant trees. We do not know, however, if this observed difference is due to active preference for ant trees by moths or is a result of higher levels of survival of moths in ant trees.

Protection from parasitoids and predators is often considered the most important benefit for insects of ant tending (see e.g. Pierce & Mead, 1981; Bristow, 1984; Bach, 1991; Sipura, 2002). Ants protect myrmecophilous lepidopterans, such as *Glaucopsyche lygdamus,* against parasitoid wasps in exchange for excreted substances harvested by the ants (Pierce & Eastcal, 1986). Untended lycaenid larvae are more than twice as likely to be parasitized as ant tended larvae (Pierce & Mead, 1981; Pierce & Easteal, 1986; Weeks, 2003). Thomas & Elmes (1993) found that ants aggressively attacked hymenopteran parasitoids attempting to enter the ant nest to infect the lycaenid larvae tended by the ants. We suggest that by restricting predator numbers and hunting areas, ants can also favour untended insect species such as psychid moths. In our study sites, the number of parasitoids was lower on ant-trees than on non-ant trees even if this result was not statistically significant due to the low total number (22) of parasitoids observed during the study period. In the third behavioural experiment (Fig. 3c.), ants attacked parasitoids while psychid larvae within their larval case remained untouched. If ants decrease the parasite risk, this would be a huge benefit since parasitoids most likely offer the greatest mortality risk to the moths. In some habitats, up to 100% of moths are parasitised (Kumpulainen et al. in press).

The spruce forest component (indicating a typical, older, shaded, spruce forest with abundant moss vegetation and minor temperature variations) correlated positively with the proportion of sexual *S. rupicolella* of moth populations. This may suggest that *S. rupicolella* prefers older forest habitats than does *D. fennicella.* The preference is not, however, exclusive since both species co-occur in several different habitat types. Moreover our northern study sites were young birch forests and still sexual *S. rupicolella* was the dominant species of Psychidae moth. Parthenogenetic moths are more common in young and mid-aged forests because they are more effective in reproduction and colonizing new habitats than sexuals. Along with forest succession, the amount of sexual species may have increased and later parasitoids have followed. In our previous studies, we found that the proportion of sexually reproducing moths correlated positively with the prevalence of parasitoids. In locations were parasitoids were rare, asexuals were dominant and vice versa (Kumpulainen et al. in press). In this study, we found the prevalence of parasitoids as well as sexual reproduction more common in the older forest habitats. Thus, one explanation for the observed correlation between old forest and sexual reproduction may be that the sexuals have outcompeted asexuals from these locations.

In conclusion, the most intensively patrolled ant trees are likely to serve as relatively safe feeding and pupating sites for psychid moths, because wood ants reduce the numbers of parasitoids and other arthropods. Ants are unlikely to pose a threat to psychid moth larvae as long as the larvae manage to remain inside their larval case. According to our knowledge, our results are the first to indicate indirect positive effects of ant predation on untended Lepidoptera larvae and on any animals that are potential, and here proved to be suitable, food for the co-existing and abundant wood ants.

Acknowledgements

We want to acknowledge K. Kulmala in particular for assisting in field work. We also want to thank K. Huhtala, R. Högmander, M. Myllylä and S. Palmu for their assistance in the field and laboratory work, J. Suutari for her help in the measurements of habitat characteristics and S. Paukku, S. Suonpää, M. Björklund and P. Mutikainen for their valuable comments on the manuscript. M. Iversen kindly corrected the language. This study was done at the University of Jyväskylä and was financially supported by the Academy of Finland, project number 779874.

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TABLE 1 Correlations between principal components (PC) and (a) ant variables, (b) location descriptive variables.

a

Variable	**Principal component**
Percentage of total variance explained	80.56
Average number of ants per tree	0.87
Average number of ant nests per location	0.89
Average number of ant paths per location	0.92
Ant activity classification	0.92

b

Variable	**PC 1**	**PC 2**
Percentage of total variance explained	50.89	20.08
Percentage of spruce / all trees	0.82	0.18
Moss coverage	0.70	0.51
Tree top coverage	0.70	-0.47
Temperature SD	-0.53	0.67
Age of forest	0.77	0.22

TABLE 2 Total quantities of collected arthropod material was tested with repeated measures ANOVA. In the model, mean quantities of arthropods (after ln-transformation) in ant trees versus non-ant trees (n= 36) were used as the dependent factor and the location (n= 6) was used as the independent variable. No interactions between the locations and quantities of arthropods between ant trees and non-ant trees were found (all p values were > .125). The number of parasitoids between ant trees and non-ant trees was compared using Wilcoxon´s test.

	Mean for ant trees	**Mean for non-ant trees**	**SE for ant trees**	**SE for non-ant trees**	**F**	**P**
Small psychid larvae	49.72	43.75	5.71	4.93	1.27	0.269
Large psychid larvae	15.42	9.31	3.33	2.10	10.8	0.003
Aphids	47.89	22.22	11.24	2.92	10.6	<0.001
Hymenopteran parasitoids	0.17	0.44	0.75	1.35		0.102
Ants	302.31	38.75	45.42	7.43	119.2	<0.001
Predatory arthropods	19.25	22.22	2.01	2.00	2.97	0.095
Other Lepidoptera larvae	6.03	9.00	5.05	8.39	10.6	0.003
Ant food Animals	12.33	17.14	1.72	1.86	5.43	0.027

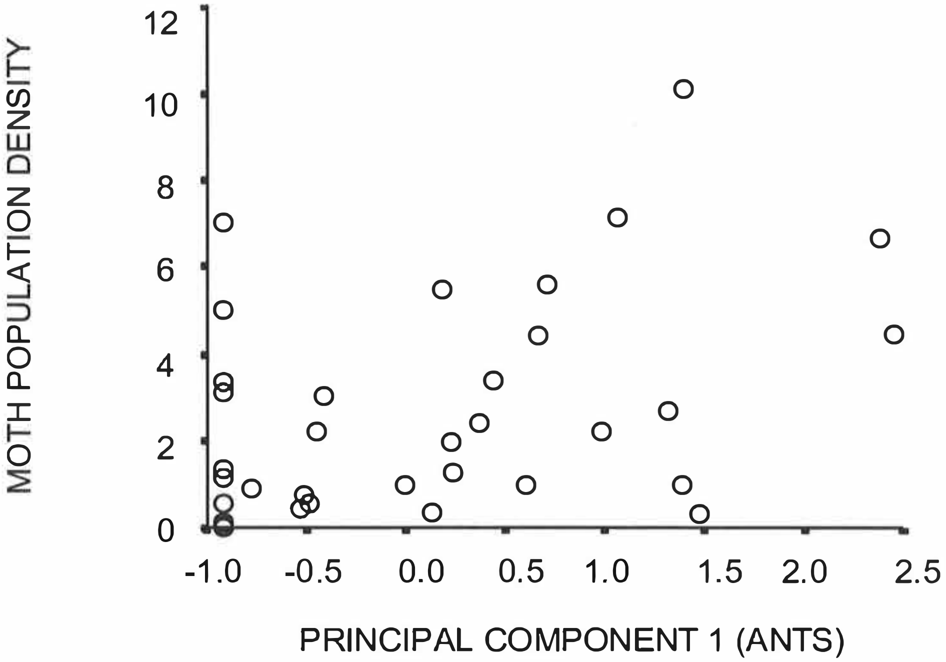

FIGURE 1a Principal component 1 (ant abundance and activity) plotted against moth population density.

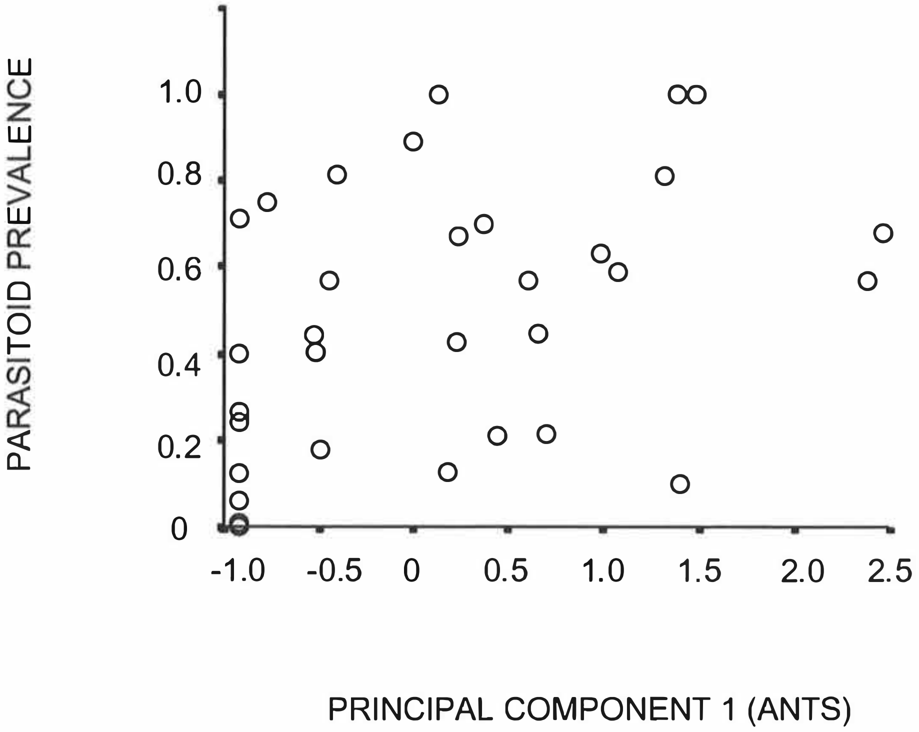

FIGURE 1b Principal component 1 (ant abundance and activity) plotted against parasitoid prevalence. Each circle represents a separate location.

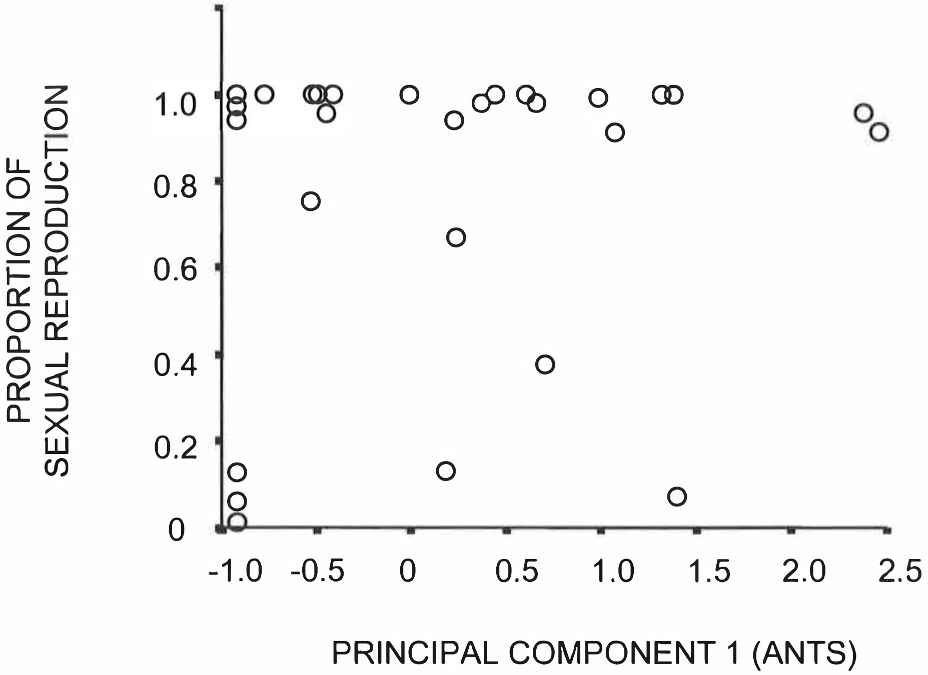

FIGURE 1c Principal component 1 (ant abundance and activity) plotted against proportion of sexual reproduction. Each circle represents a separate location.

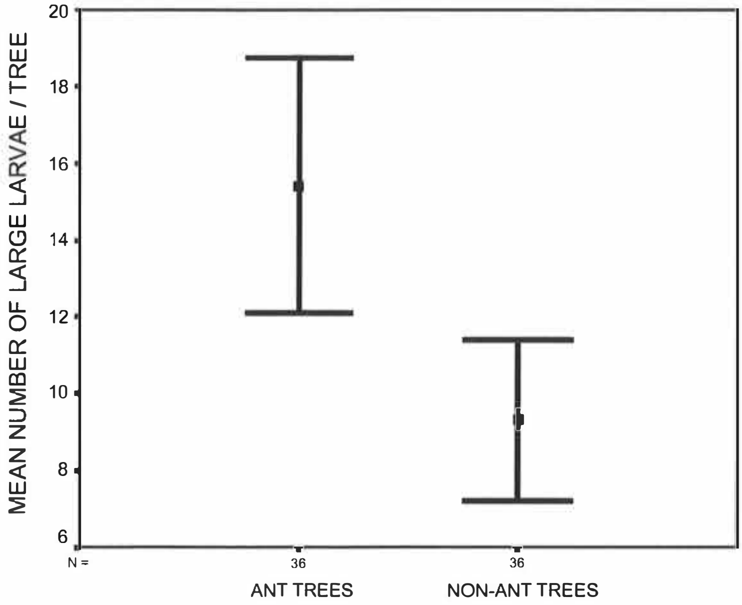

FIGURE 2a Difference between ant trees and non-ant trees in mean (±SE) number of large psychid larvae per tree.

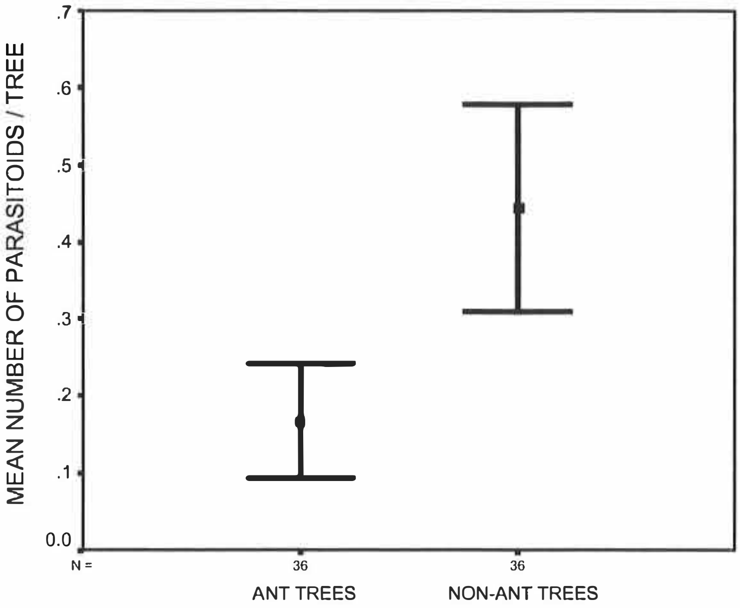

FIGURE 2b Difference between ant trees and non-ant trees in mean (±SE) number of parasitoids per tree.

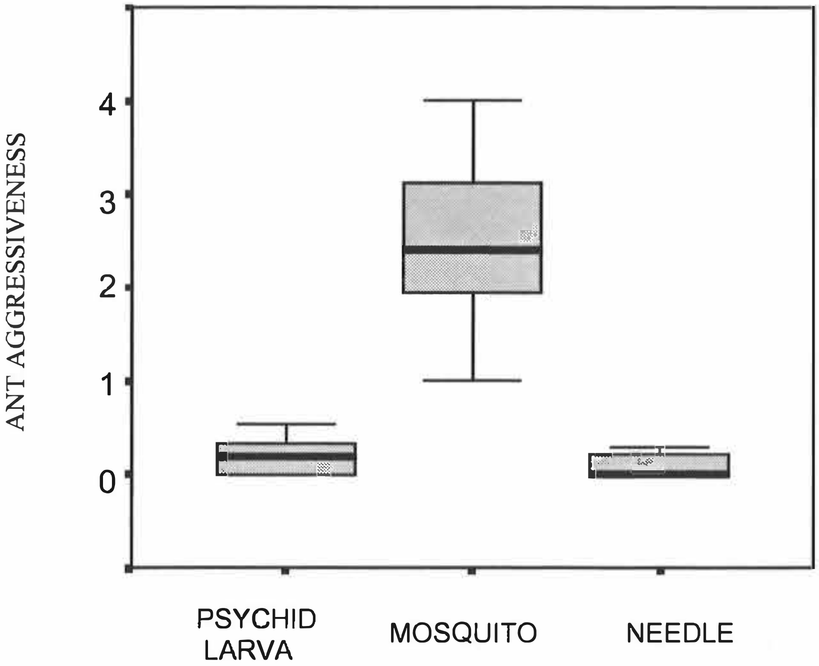

FIGURE 3a Differences in ant aggressiveness towards psychid larvae, mosquito and spruce needle.

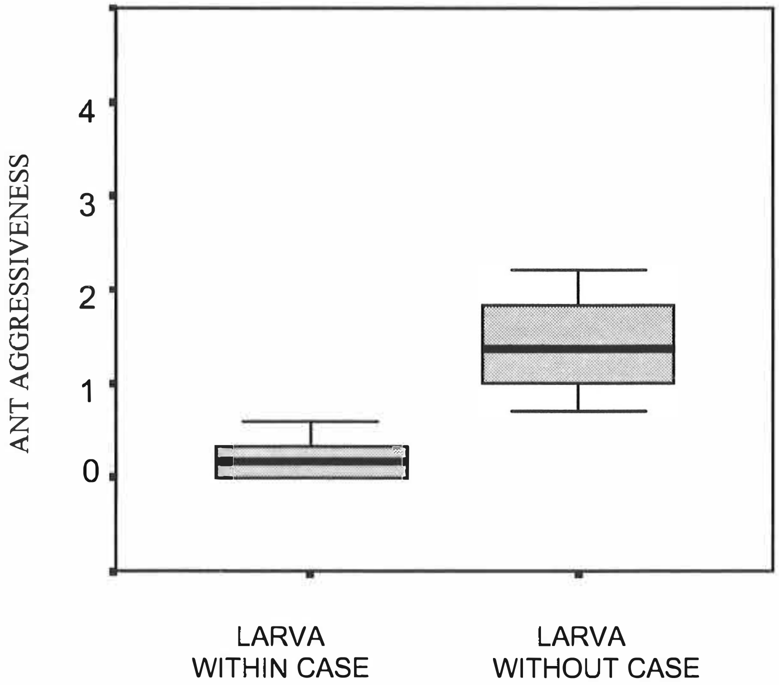

FIGURE 3b Differences in ant aggressiveness towards psychid larvae within or without larval case.

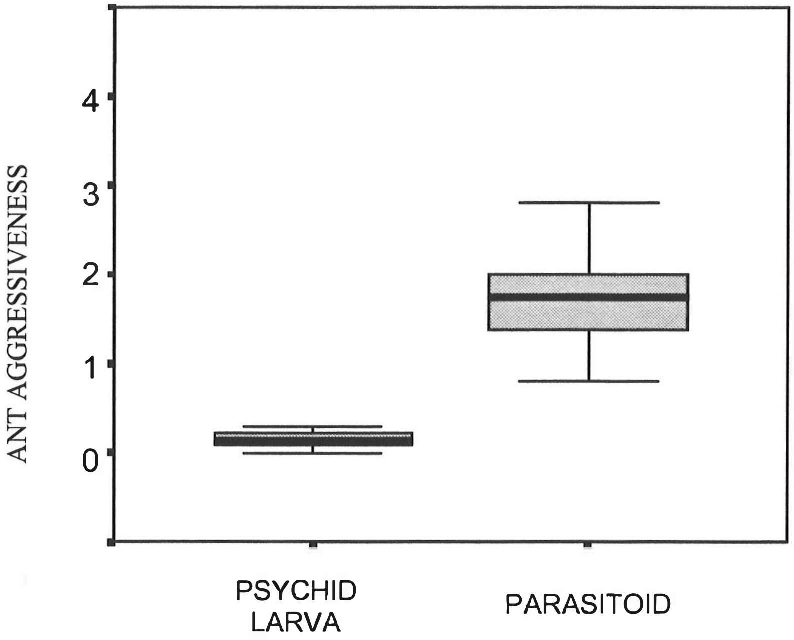

FIGURE 3c Differences in ant aggressiveness towards psychid larvae and hymenopteran parasitoids.