PARASITES AND SEXUAL REPRODUCTION IN PSYCHID MOTHS

Tomi Kumpulainen,[1,2] Alessandro Grapputo,[1,3] and Johanna Mappes[1,4]

[1]*Department of Biological and Environmental Science, PO Box 35, University of Jyväskylä, FIN-40014 Finland*
[2]*E-mail: tokumpul@bytl.jyu.fi*
[3]*E-mail: grapputo@cc.jyu.fi*
[4]*E-mail: mappes@bytl.jyu.fi*

Abstract.—Persistence of sexual reproduction among coexisting asexual competitors has been a major paradox in evolutionary biology. The number of empirical studies is still very limited, as few systems with coexisting sexual and strictly asexual lineages have been found. We studied the ecological mechanisms behind the simultaneous coexistence of a sexually and an asexually reproducing closely related species of psychid moth in Central Finland between 1999 and 2001. The two species compete for the same resources and are often infected by the same hymenopteran parasitoids. They are extremely morphologically and behaviorally similar and can be separated only by their reproductive strategy (sexual vs. asexual) or by genetic markers. We compared the life-history traits of these species in two locations where they coexist to test predictions of the cost-of-sex hypothesis. We did not find any difference in female size, number of larvae, or offspring survival between the sexuals and asexuals, indicating that sexuals are subject to cost of sex. We also used genetic markers to check and exclude the possibility of *Wolbachia* bacteria infection inducing parthenogenesis. None of the samples was infected by *Wolbachia* and, thus, it is unlikely that these bacteria could affect our results. We sampled 38 locations to study the prevalence of parasitoids and the moths' reproductive strategy. We found a strong positive correlation between prevalence of sexual reproduction and prevalence of parasitoids. 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 alone does not explain the results. We suggest that the parasite hypothesis for sex may offer an explanation for the persistence of sexual moths in this system.

Key words.—Bag worm moth, cost of sex, parasite hypothesis, parthenogenesis, Red Queen hypothesis, *Wolbachia*.

Maintenance of sexual reproduction has been a puzzling question in the theory of evolution since it was first raised by Williams (1975), Maynard Smith (1978), and Bell (1982). Many hypotheses have been presented to explain the maintenance of sex (e.g., Kondrashov 1993; Hurst and Peck 1996; Lively 1996; Jokela et al. 2003), since the asexually reproducing morphs could produce offspring more effectively than the sexuals by avoiding the costs of producing males. This cost of sex should finally lead to replacement of sexuals 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 of sexual reproduction (e.g., Lively 1996). Such short-term advantages could arise from the genetic recombination in cross-fertilization, which purges deleterious mutations and increases the amount of genetic variation (Muller 1964; Kondrashov 1988; Rice 2002).

The importance of variation in the host genotype was further developed in the parasite hypothesis (Hamilton 1980), which emphasizes the effects of coevolution of host and parasite. These coevolutionary interactions, together with strong frequency-dependent selection by high parasite prevalence, could favor variability in host genotypes, and thus rare genotypes might avoid infections because parasites are most likely to track the most common genotypes (Hamilton 1980; Ooi and Yahara 1999; Lively and Dybdahl 2000). Such coevolution can lead to continuous changes in the frequency of host genotypes. This idea is manifested Red Queen hypothesis (Van Valen 1973) that has recently become widely studied. Support for the parasite hypothesis for sex was recently offered by Negovetic and Jokela (2001), who found that asexual snails reproduced more successfully than sex-

uals, but in unfavorable environmental conditions, the situation was reversed.

Some recent studies have focused on the mechanisms restricting the success of facultative parthenogenesis within species capable of both sexual and 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 often focused on the success of sexuals versus asexuals (e.g., Rispe and Pierre 1998; Doncaster et al. 2000; Peck and Waxman 2000), but the actual paradox of the maintenance of sex still remains. Apart from the outstanding studies on New Zealand freshwater snails (Lively 1992; Fox et al. 1996; Jokela et al. 1997; Howard and Lively 1998), there is very little empirical data from natural systems where sexually and asexually reproducing morphs of nonhybrid origin coexist and compete with each other (snail, *Campeloma limum*: Johnson 2000; flatworm, *Schmidtea polychroa*: Michiels et al. 2001).

In many arthropod species, asexuality is, in fact, not a true reproductive strategy but caused by a feminizing 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 (Bourtzis and O'Neill 1998). However, these effects may be considered as true costs of producing males as the asexually reproducing morphs rarely carry the harmful effects of these bacterial infections (Bourtzis and O'Neill 1998; Kageyama et al. 1998).

Data on natural systems in which the effects of parasites on their hosts are extremely severe is even more limited. While some authors argue that it could be parasites or pathogens causing serious reduction in the fitness of their hosts

together with moderate rates of mutation that would most likely favor sexual reproduction (e.g., Howard and Lively 1994, 1998), studies have been made mainly with less harmful parasites (e.g., Hanley et al. 1995; Michiels et al. 2001). More empirical data to support any of the hypotheses around the maintenance of sex are clearly needed.

Bag worm moths (Lepidoptera: Psychidae) provide a good opportunity to investigate the coexistence of sexual and asexual reproduction in the same location. In Lepidoptera, parthenogenetic reproduction is very rare. However, in the family Psychidae and especially among *Dahlica* species, parthenogenesis seems to have evolved several times (T. Kumpulainen, A. Grapputo, and J. Mappes, unpubl. ms.), and thus several species with strictly parthenogenetic reproduction exist in this group (see Hättenschwiler 1997). Many of these species occur in Finland and often coexist in the same habitats with sexual species. The taxonomic relationships of this group are still unclear and poorly studied. Previously, all *Dahlica* and *Siederia* moths were classified in the genus *Solenobia*. So far, taxonomic classification of these two genera has been based on morphological characters (the length of a small spine in male front leg; see Hättenschwiler 1997). Recently, we investigated the phylogenetic relationships of these species based on mitochondrial DNA markers (T. Kumpulainen, A. Grapputo, and J. Mappes, unpubl. ms.) and found that sexually reproducing *S. rupicolella* and asexual *D. fennicella* are closely related but clearly different species (Suomalainen 1980). According to our molecular data, the presence of these two genera is still questionable. Here we use current classification to indicate the two species.

Apart from the reproductive mode, we could not find any reliable differences in morphology, ecology, or behavior between the sexual and asexual forms. In central Finland asexual *D. fennicella* and sexual *S. rupicolella* coexist in many locations. Some locations have only sexually reproducing species and others have only asexuals, but in most of the locations both reproductive strategies coexist. The coexistence of closely related sexual and asexual moths and the extremely heavy selective pressure caused by lethal hymenopteran parasitoids offers a good system to compare the competitive abilities of the reproductive groups, as well as to study the possible ecological factors behind the coexisting strategies.

The purpose of this study is to obtain much needed empirical data of coexisting and competing sexuals and asexuals as well as to scrutinize the two important predictions generally linked with the maintenance of sex. First, we studied whether the sexually reproducing group has costs from producing males compared to the strictly asexual reproduction in the parthenogenetic group, as the cost-of-sex hypothesis suggests (Maynard Smith 1978). There is also the possibility of sexually transmitted bacteria causing lower survival in sexually reproducing individuals and their offspring (Bourtzis and O'Neill 1998). Thus, we tested for *Wolbachia* bacteria to rule out the possibility of parthenogenesis or other reproductive features being influenced by this bacteria. Second, we examined whether strong selective pressures such as high parasitoid prevalence could favor sexually reproducing species, which is the basic assumption of the parasite hypothesis for sex (e.g., Hamilton 1980; Jokela et al. 2003). To be able

to investigate this hypothesis, the two species should coexist, compete, and be infected by the same parasites.

MATERIALS AND METHODS

Study System

In Europe *Siederia* and *Dahlica* species (Lepidoptera: Psychidae) are common, and many species have a wide distribution ranging from central Europe to northern Scandinavia. However, the status of many species is still uncertain, and species determination is mainly based on a few morphological features (see Suomalainen 1980; Hättenschwiler 1997; Kumpulainen et al., unpubl. ms.). At least three separate parthenogenetic species have been identified in the subfamily Solenobiinae, and all appear to be tetraploids, probably originating from different sexual species in the same subfamily (Suomalainen 1980; Hättenschwiler 1997; Kumpulainen et al., unpubl. ms.). Although the origin of parthenogenesis in *Dahlica* and *Siederia* species is not known, it is unlikely to be interspecific hybridization. A close relative, the parthenogenetic *D. triquetrella*, reproduces by automictic parthenogenesis. In this type of parthenogenesis the zygoid phase is restored by the fusion of the two central nuclei, which results in the formation of the Richtungs-Kopulations-Kern (RKK) structure. The fusion leads to heterogamy, which explains why only females are produced. The RKK is also formed in the sexual form of *D. triquetrella* but is not developed further and the zygoid phase is restored through the introduction of the sperm (see Suomalainen 1962). Many *Dahlica* and *Siederia* species are not only morphologically uniform, but also share most of their ecological and biogeographical features. In Finland, they occur mostly in older wooded habitats, especially in sparse forests or warmer forest edges. Most species occur patchily, and the same habitats are often occupied by several species.

Bag worm moth larvae always carry a case (or bag) made of plant material, sand, and other dry matter. Larvae feed on moss and algae and finally pupate within their larval case. Females of these 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 after egg laying. Parthenogenetic females, instead, do not excrete pheromones but start to lay eggs shortly after hatching. Males are always winged and search for newly hatched females, guided by female pheromones, but males have very limited dispersing ability and can only fly for short distances (10–100 m). The length of an adult female of both species is on average 3–5 mm and the wingspan of male on average 13–16 mm. Larvae are about the size of a female and the larval case averages 6–8.5 mm. Life cycle from egg to adult takes from one to two years, but the adult stage takes only three to six days (Suomalainen 1980). Hence, a moth spends most of its life cycle as a larva.

Psychid larvae are often infected by at least two common species of hymenopteran parasitoids (see Hättenschwiler 1997; P. Hättenschwiler, pers. comm.), including *Orthizema* spp. and a few rare species (Hymenoptera: Ichneumonidae). In all parasitoid species, females normally lay one egg per host larva. The parasitoid egg remains dormant until the fully
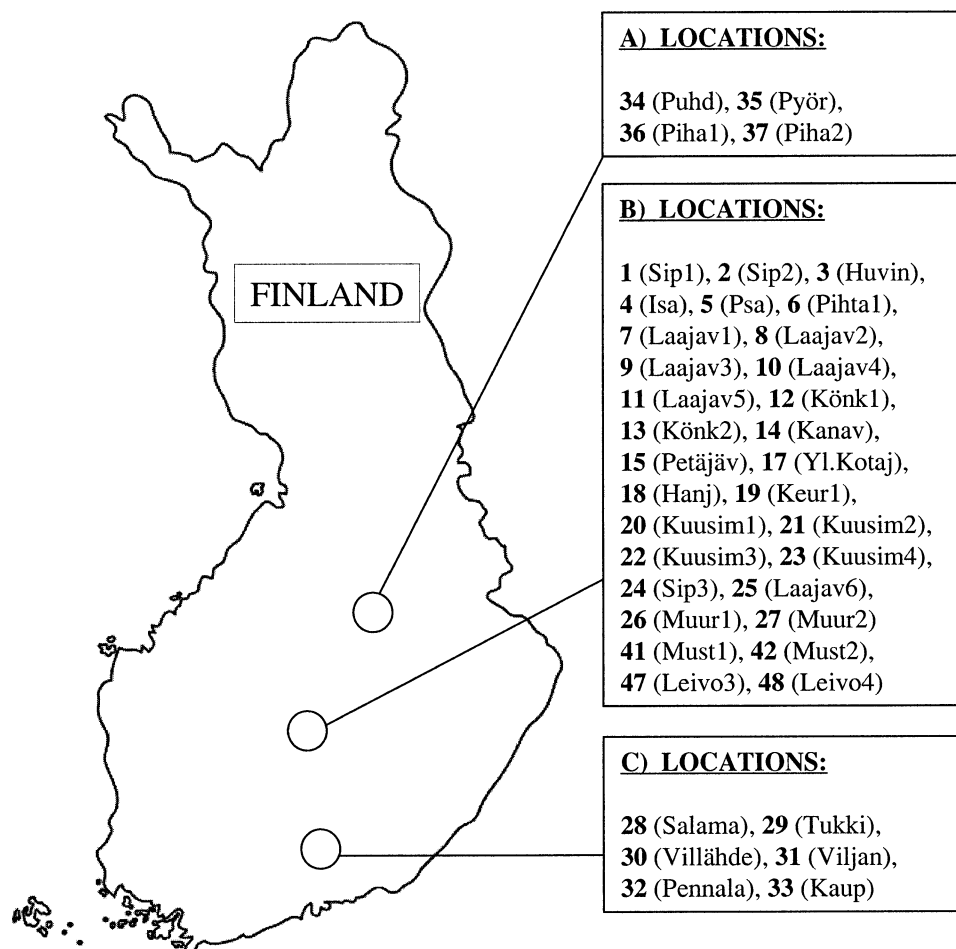
FIG. 1. Map of Finland. (A) Area of the northern populations, locations 34–37; (B) the main study area in Central Finland, locations 1–27, 41–48; (C) area of the southern populations, locations 28–33.

grown host larva pupates, when the parasitoid larva quickly eats the host, pupates, and hatches just a few weeks after the moth's hatching period. During larval development, these hymenopteran parasitoids kill their hosts—hence the cost of being parasitized is extreme. Because the infected individuals are always lost, their reproductive strategy or species cannot be determined. In preliminary laboratory experiments, we offered different host larvae 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). On the basis of previous observations, we knew that both the parasitoid prevalence and the proportion of sexuals versus asexuals vary strongly between the locations under study. *S. rupicolella* (sexual) and *D. fennicella* (asexual) are locally the most common species (80% or more of all species of psychid moths).

Cost of Sex

Individuals for the laboratory studies of cost of sex were collected from two locations in 1999, Sip 1 and Huvin, in Jyväskylä, Central Finland (62°15′N, 25°43′E; see Fig. 1,

Appendix). Both asexuals and sexuals are abundant in these locations. Study areas consisted of older forest patches, separated by lakes and fields and sometimes by human settlements. Forests are dominated by Norwegian spruce (*Picea abies*) and silver birch (*Betula pendula*), many of them also containing Scots pine (*Pinus sylvestris*) and occasionally a few European aspen (*Populus tremula*) or grey alder (*Alnus incana*). Individuals were collected in April of 1999, 2000, and 2001. To catch final instar larvae, which climb on tree trunks to pupate in early spring, we set tape traps on tree trunks where larvae get stuck and can be later collected. At Sip 1 and Huvin, we set 138 and 83 tape traps, respectively (see Appendix). The traps were set before the beginning of the climbing period (30 March to 7 April), and they were checked twice per week until the climbing period was over (30 April). Each larva was separately labeled 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, sexual females, and asexual females be-

gan to hatch. The reproductive strategy of female moths was determined on the basis of female behavior, that is, whether the females waited for males or started to lay eggs without copulation. After hatching, all females climbed on the top of their own larval case. Parthenogenetic females immediately started to lay eggs inside their larval case, whereas sexual females began to release pheromones to attract males and did not start egg laying without copulation.

We offered a randomly chosen male to each sexual female. Males quickly began flying toward the female and copulation normally succeeded immediately and lasted between 5 and 30 min. After copulation, sexual females laid their eggs inside their own larval case. Because females die within few hours of egg laying, they are only able to copulate and reproduce once. Egg clutches laid by individual females were kept separate. The number and proportion of hatching larvae were calculated.

After egg-laying, sexual and asexual females from Huvin (location 3) and Sip 1 (location 1) were individually weighed to the nearest 0.1 mg using a HA-202M microbalance (A and D Instruments, Oxford, U.K.) We measured the length of females, pupae, and larval cases. Of all female measurements, the pupal length proved to be the most reliable measurement of female size, and thus it was used to indicate female size in our study.

F_1 clutches produced by sexual and asexual females were grown in laboratory conditions (19 ± 2°C, 80% humidity, food ad libidum) similar to their natural habitats. The survival rates of moth larvae were checked before the overwintering period. Because females of sexually and asexually reproducing species are practically impossible to separate reliably before their reproduction mode, we were forced to randomly collect as many individuals per location as possible. Because the adults are very short lived (maximum three to six days) and only males hatching on the same day as females could be successfully mated, there were only a few, if any, suitable mate choices at a time; therefore, sample sizes of reproductive success for sexuals remained lower than for asexuals.

Wolbachia Analysis

Total genomic DNA was extracted from whole single adult females of sexual *S. rupicolella*, parthenogenetic *D. fennicella*, and their hymenopteran parasitoids in 1999 using the Chelex extraction method (Walsh et al. 1991). Eighty-five females (44 parthenogenetic and 41 sexual) and 10 parasitoids were tested for the presence of the bacteria *Wolbachia pipientis* via polymerase chain reaction (PCR). These samples were collected randomly from several study populations. Three sets of primers were used to amplify two *Wolbachia* genes, wsp (the gene encoding the major surface protein) and 16S rDNA. The general primers wsp 81F and 691R (Braig et al. 1998), which are able to amplify both major strains of *Wolbachia* (A and B), were used to amplify approximately 600 bp of the wsp gene. The primers 16SAf + 16SAr and 16SBf + 16SBr, specific to the *Wolbachia* strains A and B, respectively, were used to amplify approximately 250 bp of the 3′ portion of the 16S rDNA.

PCR was performed in a total volume of 25 μl using 10 mM of Tris-HCl, 1.5 mM MgCl$_2$, 5 pmoles of each primer,

400 μM of each dNTPs, and 0.5 units of Taq polymerase (Gibco-BRL, Gaithersburg, MD). PCR cycling conditions were one cycle at 94°C for 5 min, followed by 30 cycles of 30 sec at 94°C, 30 sec at 50°C, and 1 min at 72°C followed by one cycle at 72°C for 5 min. The PCR products were resolved in a 1% agarose gel stained with ethidium bromide. Naturally infected ants, *Gnamptogenys menadensis*, were used as a positive control, and uninfected ants, *G. moelleri*, were used as a negative control in each amplification. To check if DNA extraction was successful, PCR was also preformed using primers S2792 and A3389 (Simon et al. 1994), which amplify a fragment of the mitochondrial COII gene.

Parasitoid Prevalence and Proportion of Sexual Reproduction

Samples for parasitoid prevalence studies were collected from similar habitats as the moths for the cost-of-sex studies (see above). However, prevalence of parasitoids in the study species in 1999 was determined in 15 different study locations within a relatively small area (15 km × 30 km; see Appendix) around Jyväskylä, Central Finland (62°15′N, 25°43′E: see Fig. 1). In the following two years, 2000 and 2001, the parasitoid prevalence and the proportion of sexual reproduction were determined in 29 and 19 locations, respectively (for reproductive strategy determination, see the cost-of-sex discussion above). In 2000, we also sampled additional areas in southern Finland, near Orimattila, and in the northern part of Central Finland, around Sonkajärvi (see Appendix and Fig. 1 for exact locations). In each area, we set five to 138 tape traps (see Appendix). In smaller forest patches, practically all trees were tape trapped, whereas in larger forest patches trunks were chosen randomly. Location 4 (Isa) was exceptional in 1999, since all bag worms of this population were collected from the walls of a house and, thus, they were hand picked. Sample sizes are presented in the Appendix.

Although some of the study locations are close to each other (in certain cases less than 200 m), we treated them as separate populations since preliminary genetic data of an ongoing study (A. Grapputo, T. Kumpulainen, and J. Mappes, unpubl. ms.) shows very low gene flow between neighboring populations. Moreover, females do not disperse and males can fly only very short distances. In our study system, reproductive strategy results are based on moth individuals not infected by parasitoids, as infected individuals are always lost and neither their reproductive strategy nor their species can be determined.

In the laboratory, reproductive mode and sex of the hatching moths was monitored. Approximately four weeks after the adult moths' hatching period (two months after collecting the larvae), the parasitoids started to hatch from the moth pupa, which had been 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. Our study species are infected by several species of Hymenopteran parasitoids belonging mostly to genus *Orthizema*. Here the parasitoid prevalence means total parasitoid load and thus covers all the hymenopteran parasitoids hatching from the collected host moths. The effects
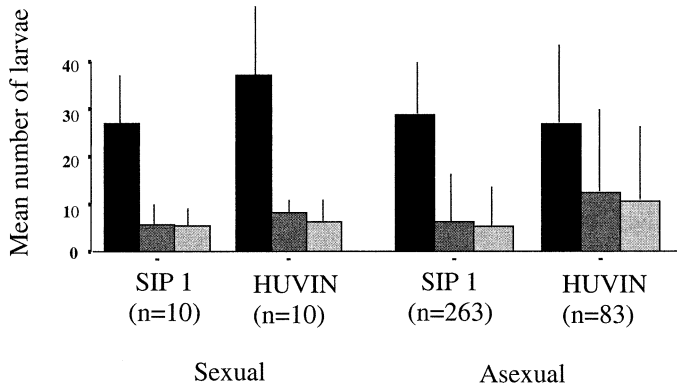
FIG. 2. The number of larvae at hatching (black bars) and surviving larvae after one month (gray bars) and in fall (white bars). Vertical lines show standard deviations.

of habitat characteristics (size, location, and main tree species) on the proportion of sexual reproduction in each location were also analyzed.

RESULTS

Cost of Sex

Comparison of pupal length measurements did not reveal any significant differences in size between the females of sexual *S. rupicolella* and asexual *D. fennicella* (two-way ANOVA for ranked data: $F_{1,358} = 0.567$, $P = 0.452$) or between the two locations ($F_{1,358} = 0.855$, $P = 0.356$). We did not find any significant differences between species in the number of hatched larvae nor in the number of larvae that survived until the following autumn (ANOVA for ranked data: $F_{1,358} = 0.017$, $P = 0.897$; $F_{1,358} = 0.215$, $P = 0.643$) or between locations ($F_{1,358} = 0.904$, $P = 0.342$; $F_{1,358} = 3.40$, $P = 0.066$; see Fig. 2). Sexuals had only 0.37% higher reproductive output (number of hatched larvae) at Sip 1 (location 1) when compared to asexuals. At Huvin (location 3) asexuals had 3.2% higher reproductive output (Fig. 2). However, we found a significant difference in hatching date between asexuals and sexuals. Asexuals tended to hatch on average five days earlier than sexuals at Sip 1 and six days earlier at Huvin (Mann-Whitney U-test, $Z = 2.4$, $n_1 = 10$, $n_2 = 263$, $P = 0.018$; $Z = 3.2$, $n_1 = 10$, $n_2 = 83$, $P = 0.002$, respectively).

Wolbachia

No infection from *W. pipientis* was detected in females of either species. None of the genes (wsp and 16S rDNA) were detected by amplification, whereas 90% of the parasitoids were infected with both A and B strains of *Wolbachia*.

Parasitoid Prevalence and Proportion of Sexual Reproduction

The proportion of sexually reproducing females (of all hatched females) varied from 0% to 100% among the 38 study locations. The proportion of sexually reproducing females in these locations was significantly and positively correlated with the proportion of moth larvae infected by hymenopteran parasitoids ($r_{s1999} = 0.802$, $N = 15$, $P < 0.001$; $r_{s2000} = 0.542$, $N = 29$, $P = 0.002$; $r_{s2001} = 0.811$, $N = 19$, $P < 0.001$). This may suggest that sexual reproduction could compensate for the costs of sex in environments with heavy parasitoid loads (Fig. 3). Although sexuality/asexuality seems to be strongly clustered, geographic distribution does not explain the occurrence of the reproductive strategies (Mantel's test: $r_{1999} = 0.04$, $P = 0.712$; $r_{2000} = 0.05$, $P = 0.554$; $r_{2001} = 0.11$, $P = 0.094$). The size of the habitat patches did not correlate with prevalence of parasitoids in any year (all $P > 0.600$). The density of populations (individuals per trap) varied from 0.1 to 34 among locations. There was a strong correlation in 2000 ($r_{s2000} = -0.64$, $N = 29$, $P < 0.001$) and a nonsignificant tendency in 1999 ($r_{s1999} = -0.52$, $N = 14$, $P = 0.056$) for prevalence of parasitoids to correlate negatively with host density. In 2001, with the smallest sample size, there was no correlation ($r_{s2001} = -0.045$, $N = 13$, $P = 0.170$). This indicates that in locations with high parasitoid loads host populations are sparse. The proportion of sexuals did not correlate with population density ($r_{s1999} = -0.29$, $N = 14$, $P = 0.300$; $r_{s2000} = -0.157$, $N = 29$, $P = 0.416$; $r_{s2001} = -0.44$, $N = 12$, $P = 0.148$). To maintain reliability of these analyses, sample sizes smaller than 10 individuals per location were excluded from the Spearman correlations when analyzing proportion of sexuality in locations.

DISCUSSION

We found that sexual and asexual females have equal reproductive output, which indicates that sexuals may suffer from the cost of sex (Maynard Smith 1978) as half of their offspring will be males (see Fig. 2).

Most interestingly, we found a strong positive correlation between parasitoid prevalence and the occurrence of sexual reproduction in three years (Fig. 3). This means that sexually reproducing moths (*S. rupicolella*) are usually abundant in locations where lethal hymenopteran parasitoids are common, while asexuals (*D. fennicella*) dominate where the parasitoid prevalence is lower. However, due to the nature of our study system, we are not able to compare the reproductive strategy of infected versus noninfected moths because the infected individuals are always lost and their reproductive strategy or species therefore cannot be determined. Thus, our results on reproductive strategy are based on moth individuals uninfected by parasitoids.

In recent publications, the foremost hypothesis to explain the evolution of sex is the parasite hypothesis for maintenance of sex, which relies on coevolutionary interactions between host and parasite (Hamilton 1980) and particularly on genetic variability in resistance to the parasite (e.g., Grosholz 1994). The parasite hypothesis states that coevolutionary interactions between host and parasite, together with strong frequency-dependent selection caused by the parasites, could favor variability in host genotypes, 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 favor sexuals with a higher recombination rate unless the number of asexual clones is very high (for discussion about the hy-
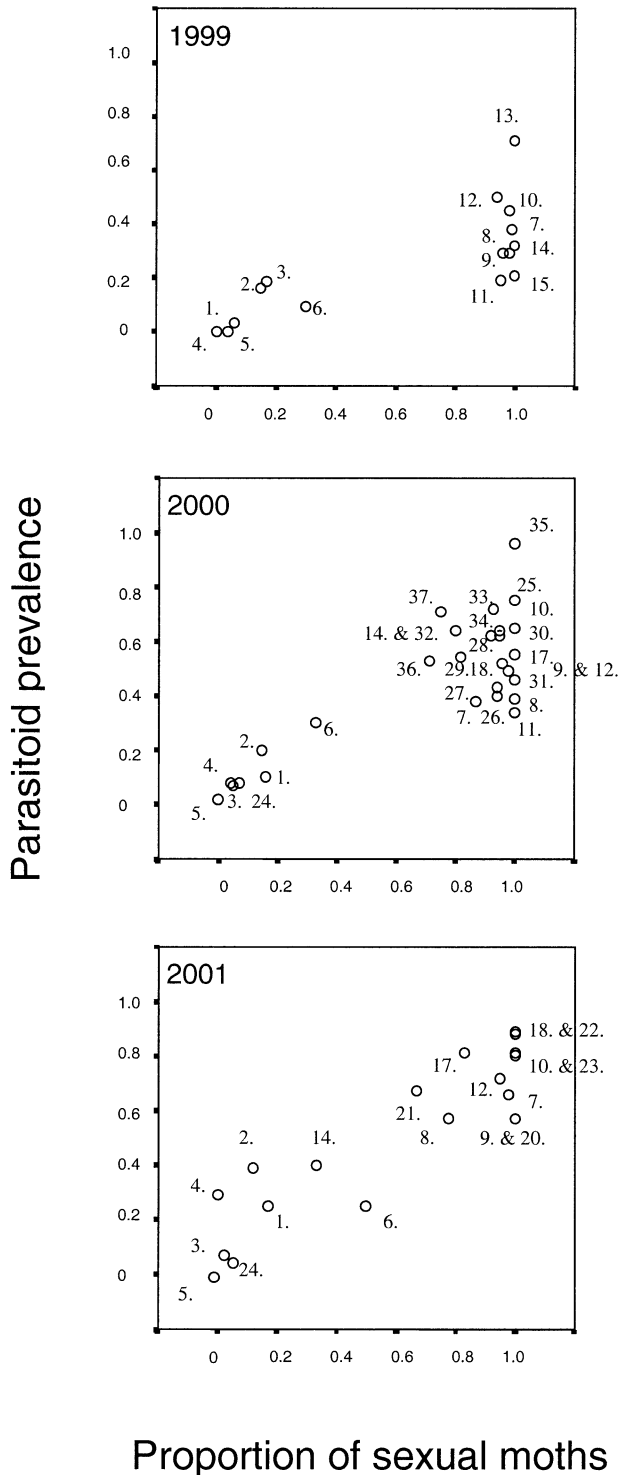
FIG. 3. Correlation between parasitoid prevalence and the proportion of sexuality (A) in 1999 at 15 locations; (B) in 2000 at 29 locations; and (C) in 2001 at 19 locations. Names and descriptions of the study locations are given in the Appendix.

potheses, see Hamilton 1980; Lively 1992, 1996; Greig et al. 1998; Jokela et al. 2003).

Our result appears to support the parasite hypothesis, since both sexual and asexual moth species are frequently infected

by the common hymenopteran parasitoids (Hättenschwiler 1997; P. Hättenschwiler, pers. comm.). Moreover, our own field and laboratory observations (1999–2002) indicate that parasitoids do not discriminate between the larvae of different host species as long as the larvae are fully grown and have not been previously infected by other parasitoids.

The general value of the parasite hypothesis has been highlighted by previous studies on at least two different freshwater snail species (Lively 1987, 1992; 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). In our study system, the evolutionary forces between the psychid moths and their parasitoids may well give rise to an advantage for sexual reproduction, if the coadapted hymenopteran parasitoids are able to infect the most common host genotypes and if the asexual hosts have less genetic variability than the sexuals (Hamilton 1980; Hurst and Peck 1996; Lively 1996). In such a situation, only a small proportion of rare or new, and thus most likely sexually produced, genotypes could escape the parasites or other strong selective pressure (see Greig et al. 1998). Thus, parasite pressure could favor sexual reproduction if such reproduction produces more genetic variation in the population. In cases where the asexual population contains more genetic variation than the sexuals, parasite prevalence is not expected to favor sexuality (see Lively et al. 1990; Hanley et al. 1995). In general, lethal parasites are thought to have the most remarkable effects on competition between closely related sexual and asexual species (see May and Anderson 1983). Moreover among psychid moths, remarkable effects on fitness are present, as the infected individuals are excluded from the population before they mature.

Many previous empirical studies with comparative perspectives have been made between sexuals and parthenogens of hybrid origin (Vrijenhoek et al. 1989; Lively et al. 1990; Moritz et al. 1991; Radtkey et al. 1995; Schartl et al. 1995; Mantovani et al. 1996). In certain breeding systems interdependence between the reproductive strategies still exists. Among the gynogenetic fish species *Poecilia*, the parthenogenetic reproduction is dependent on the availability of males of the sexual competitors. Although the male's genome does not contribute to the next generation, the sperm is still needed to activate the development of the embryo (Hubbs and Hubbs 1932). In systems with asexual competitors of hybrid origin, comparison between the sexuals and asexuals may be difficult because the hybrids have new genetic combinations originating from two different genomes and they are often subjected to outbreeding effects such as elevated heterozygocity levels (see Jokela et al. 1997).

In our study, the parthenogenetic moth is most likely an independent species of nonhybrid origin (Kumpulainen et al., unpubl. ms.). According to our phylogenetic studies, *S. rupicolella* and *D. fennicella* are close relatives and members of a compact phylogenetic group (Kumpulainen et al., unpubl. ms.). Previously, niche separation has been found also among nonhybrid clones of at least the moth *Alsophila pometaria* (Futuyama et al. 1981; Harshman and Futuyama 1985) and *Ips* bark beetles (Kirkendall and Stenseth 1990). However, in our system, we were not able to define the possible niche differences of the sexual and asexual moths. Furthermore,

both species were successfully grown under same laboratory conditions and with same larval host plants. This, together with all the information about the ecological and morphological similarity of our study species (see Suomalainen 1980; Hättenschwiler 1997), gives us reasonable grounds to compare the success of asexual and sexual reproduction.

If any cost of sex exists, the conditions for the all-else-equal requirement should be met. In our study, we could not find any significant differences in the reproductive output of sexual *S. rupicolella* and asexual *D. fennicella* females. If a parthenogenetic female is able to produce the same amount of offspring as a pair of sexual moths, a cost of sexual reproduction exists in sexual *S. rupicolella*. To our knowledge, this kind of cost for sexual reproduction in noncyclical insects (or other arthropod species) compared to coexisting asexual species has not been detected in earlier empirical studies with no indication of bacterial sex-ratio distortion. According to our results, no additional cost of sex caused by *Wolbachia* exists here, and the causes of parthenogenesis in *D. fennicella* are to be found elsewhere.

Many earlier studies found that the number of eggs produced or survival at larval phases is lower in asexuals than in sexuals (e.g., Bell 1982; Corley et al. 2001). In such systems, asexuality may function as a secondary reproductive strategy when sexual reproduction is not possible. In *D. fennicella*, parthenogenesis is the sole reproductive strategy and no males are known for this species. Facultative parthenogenesis is not known to exist among any solenobid psychids.

It has been shown that, with a considerable rate of mutations per genome (Keightley and Eyre-Walker 2000), mutation accumulation in the asexuals could explain why sexuals can outnumber the asexuals (Lively 1996; Peck and Waxman 2000; Rice 2002). Even though the rate of mutation per genome has not been studied in psychid moths, most asexual populations are quite small (see Materials and Methods) and hence the conditions could also be suitable for Muller's ratchet (Muller 1964) to work. However, West et al. (1999) emphasized the importance of a pluralistic view instead of finding simple explanations for the maintenance of sex in complex systems, and Howard and Lively (1998) suggested that even moderate effects of parasites together with mutation accumulation could act in favor of sex. However, in our study system it is unlikely that mutation accumulation could explain the maintenance of sexuals as the life-history measurements do not indicate any phenotypic abnormalities among the asexuals.

One interesting finding in our study was that the highest parasitoid prevalence was found in the most sparsely populated locations. This finding is quite opposite to the general expectations of the host-parasite interactions, where strong density dependence between parasitoids and hosts is expected (e.g., Begon et al. 1996). The reason for our findings is unclear, but it is possible that in the densest asexual locations prevalence of parasitoids would increase in following years. However, in four locations where asexuals dominated in 1999, the parasitoid prevalence increased in following years, whereas in one location prevalence decreased and in one location the prevalence remained the same (Fig. 3).

The observed correlation between proportion of sexuality and parasitoid prevalence could result from other factors, such as temperature or other abiotic conditions that could be unfavorable to both parasitoids and sexual reproduction in certain locations where asexuals manage to persist. Thus, our system could be a case of geographical parthenogenesis, a situation in which the sexual and the parthenogenetic relatives differ in their distribution areas, often due to some environmental conditions (Seiler 1923; Vandel 1928; Suomalainen 1950; Glesener and Tilman 1978). The logic behind geographical parthenogenesis is that asexuals do not have to mate and recombine their genome with nonoptimal mates (possibly migrants), which might not have the necessary adaptations for the environment (Peck et al. 1998; see also Maynard Smith 1978; Greig et al. 1998). Both of our study species live in presumably harsh conditions, on the edges of their distribution area (Suomalainen 1980). This is one reason why asexuals are expected to be better in colonizing new habitats and why they are expected to be found on the edges of the distribution area. However, we do not think that habitat quality alone would explain our results. Neither habitat size (Appendix) nor geographical location had a significant effect on the proportion of sexually reproducing moths in any of the three study years (see Materials and Methods). Furthermore, parasitoids are present to some extent in all study areas. The sexual and asexual moths coexist in most of the locations, and we did not find indication that sexuals and asexuals would be found preferentially in habitats that clearly differed in type, size, or structure (see Appendix). However, we cannot totally exclude the possibility that some (ecological) factor could affect the apparent correlation between the parasitoid prevalence and sexual reproduction detected in this study.

Our results suggest a connection between the occurrence of sexually reproducing *S. rupicolella* and levels of parasitoids. This means that the hymenopteran parasitoids are likely to prevent the asexual *D. fennicella* from outnumbering their sexual competitors in populations where the parasitoid prevalence is high. Without the parasitoids, the more effectively reproducing asexuals should be able to displace their sexual competitors. So, why have these parasitoids not infected all the asexuals and caused their total extinction from their distribution area? There is no simple answer, but one possible explanation may be hidden in the genetic variability of the competing species. Recently, we found that genetic variability is higher in sexual species than in asexual species but clonal diversity in asexual *D. fennicella* is still very high (A. Grapputo, T. Kumpulainen, and J. Mappes, unpubl. ms.). Thus, it is likely that clonal extinction is relatively common and a replacement process of new clones frozen from sexual populations maintains the asexual population (Vrijenhoek 1979).

Our results give rare and needed empirical information of a novel system where a sexually reproducing species maintains itself among coexisting and more effectively reproducing asexual competitors. Overall, this result shows that in systems suitable for studying the evolution of sex, species boundaries should not prevent the comparison of asexual and sexual reproduction if these reproductive morphs are of common ancestry and still share most of their ecological features.

In summary, our results indicate that there are costs of reproducing sexually among *Siederia* moths and that these costs are not caused by microorganisms such as *Wolbachia*.

Our results also indirectly suggest that the severe costs of lethal parasites on asexual reproduction could help to maintain sex in populations of both reproductive groups. Thus, our main results are in concordance with cost-of-sex hypothesis and with the parasite hypothesis for sex.

LITERATURE CITED

Begon, M., J. L. Harper, and C. R. Townsend. 1996. Parasitism and disease. Pp. 429–481 in M. Begon, J. L. Harper and C. R. Townsend. eds. Ecology: individuals, populations and communities. Blackwell, Oxford, U.K.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. Univ. of California Press, Berkeley, CA.

Bourtzis, K., and S. O'Neill. 1998. Wolbachia infections and arthropod reproduction: Wolbachia can cause cytoplasmic incompatibility, parthenogenesis and feminisation in many arthropods. Bioscience 48:287–293.

Braig, H. R., W. Zhou, S. L. Dobson, and S. L. O'Neill. 1998. Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont Wolbachia pipientis. J. Bacteriol. 180:2373–2378.

Buckling, A., and P. B. Rainey. 2002. The role of parasites in sympatric and allopatric host diversification. Nature 420: 496–499.

Corley, L. S., J. R. Blankenship, and A. J. Moore. 2001. Genetic variation and sexual reproduction in the facultatively parthenogenetic cockroach Nauphoeta cinerea: implications for the evolution of sex. J. Evol. Biol. 14:68–74.

Doncaster, C. P., G. E. Pound, and S. J. Cox. 2000. The ecological cost of sex. Nature 404:281–285.

Fox, J. A., M. F. Dybdahl, J. Jokela, and C. M. Lively. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (Potamopyrgus antipodarum). Evolution 50: 1541–1548.

Futuyama, D. J., S. L. Leipertz, and C. Mitter. 1981. Selective factors affecting clonal variation in the fall cankerworm Alsophila pometaria (Lepidoptera: Geometridae). Heredity 47: 151–172.

Glesener, R. R., and D. Tilman. 1978. Sexuality and the components of environmental uncertainty: clues from geographical parthenogenesis in terrestrial animals. Am. Nat. 112:659–673.

Greig, D., R. H. Borts, and E. J. Louis. 1998. The effect of sex on adaptation to high temperature in heterozygous and homozygous yeast. Proc. R. Soc. Lond. B 265:1017–1023.

Grosholz, E. D. 1994. The effects of host genotype and spatial distribution on trematode parasitism in a bivalve population. Evolution 48:1514–1524.

Hamilton, W. D. 1980. Sex versus non-sex versus parasite. Oikos 35:282–290.

Hanley, K. A., R. Fisher, and T. J. Case. 1995. Lower mite infestations in an asexual gecko compared with its sexual ancestors. Evolution 49:418–426.

Harshman, L. G., and D. J. Futuyama. 1985. The origin and dis-

tribution of clonal diversity in Alsophila pometaria (Lepidoptera: Geometridae). Evolution 39:315–324.

Hättenschwiler, P. 1997. Die Sackträger der Schweiz. Pp. 165–308 in Schweizerische Bund für NaturSchutz, Schmetterlinge und ihre lebensräume, Arten-Gefährung-Schutz. Band 2. Fotorotar, Egg, Switzerland.

Howard, R. S., and C. M. Lively. 1994. Parasitism, mutation accumulation and the maintenance of sex. Nature 367:554–557.

———. 1998. The maintenance of sex by parasitism and mutation accumulation under epistatic fitness functions. Evolution 52: 604–610.

Hubbs, C. L., and L. C. Hubbs. 1932. Apparent parthenogenesis in nature, in a form of fish of hybrid origin. Science 76:628–630.

Hurst, L. D., and J. R. Peck. 1996. Recent advances in understanding of the evolution and maintenance of sex. Trends Ecol. Evol. 11: 46–52.

Johnson, S. G. 2000. Population structure, parasitism, and survivorship of sexual and autodiploid parthenogenetic Campeloma limum. Evolution 54:167–175.

Jokela, J., C. M. Lively, M. F. Dybdahl, and J. A. Fox. 1997. Evidence for a cost of sex in the freshwater snail Potamopyrgus antipodarum. Ecology 78:452–460.

———. 2003. Genetic variation in sexual and clonal lineages of a freshwater snail. Biol. J. Linn. Soc. 79:165–181.

Kageyama, D., S. Hoshizaki, and Y. Ishikawa. 1998. Female-biased sex ratio in the Asian corn borer, Ostrinia furnacalis: evidence for the occurrence of feminizing bacteria in an insect. Heredity 81:311–316.

Keightley, P. D., and A. Eyre-Walker. 2000. Deleterious mutations and the evolution of sex. Science 290:331–333.

Kirkendall, L. R., and N. C. Stenseth. 1990. Ecological and evolutionary stability of sperm-dependent parthenogenesis: effects of partial niche overlap between sexual and asexual females. Evolution 44:698–714.

Kondrashov, A. S. 1988. Deleterious mutations and the evolution of sexual reproduction. Nature 336:435–440.

———. 1993. Classification of hypotheses on the advantage of amphimixis. J. Hered. 84:372–387.

Lively, C. M. 1987. Evidence from a New Zealand snail for the maintenance of sex by parasitism. Nature 328:519–521.

———. 1992. Parthenogenesis in a freshwater snail: reproductive assurance versus parasitic release. Evolution 46:907–913.

———. 1996. Host-parasite coevolution and sex. Do interactions between biological enemies maintain genetic variation and cross-fertilization? Bioscience 46:107–114.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes. Nature 405:679–681.

Lively, C. M., C. Craddock, and R. Vrijenhoek. 1990. Red Queen hypothesis supported by parasitism in sexual and clonal fish. Nature 344:864–866.

Mantovani, B., F. Tinti, M. Barilani, and V. Scali. 1996. Current reproductive isolation between ancestors of natural hybrids in Basillus stick insects (Insecta: Phasmatodea). Heredity 77: 261–268.

May, R. M., and R. M. Anderson. 1983. Epidemiology and genetics in the coevolution of parasites and hosts. Proc. R. Soc. Lond. B 219:281–314.

Maynard Smith, J. 1978. The evolution of sex. Cambridge Univ. Press, Cambridge, U.K.

Michiels, N. K., L. W. Beukeboom, N. Pongratz, and J. Zeitliger. 2001. Parthenogenetic flatworms have more symbionts than their coexisting, sexual conspesifics, but does this support the Red Queen? J. Evol. Biol. 14:110–119.

Moritz, C., H. McCallum, S. Donnellan, and J. D. Roberts. 1991. Parasite loads in parthenogenetic and sexual lizards support for the Red Queen hypothesis. Proc. R. Soc. Lond. B 244:145–149.

Muller, H. J. 1964. The relation of recombination to mutational advance. Mutat. Res. 1:2–9.

Negovetic, S., and J. Jokela. 2001. Life-history variation, phenotypic plasticity, and subpopulation structure in a freshwater snail. Ecology 82:2805–2815.

Ooi, K., and T. Yahara. 1999. Genetic variation of geminiviruses:

comparison between sexual and asexual host plant populations. Mol. Ecol. 8:89–97.

Peck, J. R., and D. Waxman. 2000. Mutation and sex in a competitive world. Nature 406:399–404.

Peck, J. R., J. M. Yerslay, and D. Waxman. 1998. Explaining the geographic distributions of sexual and asexual populations. Nature 391:889–892.

Radtkey, R. R., S. C. Donnellan, R. N. Fisher, C. Moritz, K. A. Hanley, and T. J. Case. 1995. When species collide: the origin and spread of an asexual species of gecko. Proc. R. Soc. Lond. B. 259:142–152.

Rice, W. R. 2002. Experimental tests of the adaptive significance of sexual recombination. Nat. Rev. Genet. 3:241–251.

Rispe, C., and J.-S. Pierre. 1998. Coexistence between cyclical parthenogens, obligate parthenogens, and intermediates in a fluctuating environment. J. Theor. Biol. 195:97–110.

Schartl, M., B. Wilde, I. Schlupp, and J. Parzefall. 1995. Evolutionary origin of a parthenoform, the Amazon molly *Poecilia formosa*, on the basis of a molecular genealogy. Evolution 49: 827–835.

Seiler, J. 1923. Geschlechtschromosomenuntersuchungen an Psychiden. IV. Die parthenogenese der Psychiden. Z. induckt. Abstamm.-u. VerebLehre 31:1–99.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved poly-merase chain-reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Suomalainen, E. 1950. Parthenogenesis in animals. Adv. Genet. 3: 193–253.

———. 1962. Significance of parthenogenesis in the evolution of insects. Annu. Rev. Entomol. 7:349–365.

———. 1980. The Solenobiinae species of Finland (Lepidoptera: Psychidae), with a description of a new species. Entomol. Scand. 11:458–466.

Vandel, A. 1928. La parthénogenèse géographique contribution a létude biologique et cytologique de la parthénogenèse naturelle. Bull. Biol. Fr. Belg. 62:164–281.

Van Valen, L. 1973. A new evolutionary law. Evol. Theory 1:1–30.

Vrijenhoek, R. C. 1979. Factors affecting clonal diversity and co-existence. Am. Zool. 19:787–797.

Vrijenhoek, R. C., R. M. Dawley, C. J. Cole, and J. P. Bogart. 1989. A list of the known unisexual vertebrates. Pp. 24–31 *in* R. M. Dawley, and J. P. Bogart, eds. Evolution and ecology of unisexual vertebrates. New York State Museum, Albany, NY.

Walsh, P. A., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction DNA for PCR-based typing from forensic material. BioTechniques 10:506–513.

West, S. A., C. M. Lively, and A. F. Read. 1999. A pluralist approach to sex and recombination. J. Evol. Biol. 12:1003–1012.

Williams, G. C. 1975. Sex and evolution. Princeton Univ. Press, Princeton, NJ.

Corresponding Editor: M. Björklund

APPENDIX

Description of sampling and study locations. In forest type, main genera of trees are listed first; genera in parentheses are less numerous.

Site number	Site name	Habitat size	Forest type	Number of traps			Sample size			Geographical location
				1999	2000	2001	1999	2000	2001	
1	Sip 1	50 × 60 m	*Betula (Pinus, Picea)*	138	65	8	556	480	40	62°12′25″N, 25°44′31″E
2	Sip 2	15 × 35 m	*Picea (Pinus, Betula)*	60	74	11	354	936	28	62°11′56″N, 25°45′07″E
3	Huvin	45 × 100 m	*Betula (Pinus, Picea)*	83	30	10	311	248	188	62°16′45″N, 25°42′50″E
4	Isa	20 × 25 m	*Betula, Populus, Pinus*	—	40	5	8	291	14	62°16′40″N, 25°42′45″E
5	Psa	10 × 15 m	*Betula, Pinus, Alnus*	29	11	5	80	46	16	62°16′43″N, 25°42′41″E
6	Pihta 1	25 × 30 m	*Betula, Picea*	20	40	10	117	374	16	62°16′53″N, 25°42′56″E
7	Laajav 1	10 × 40 m	*Picea, Pinus, Betula*	21	27	7	37	108	6	62°15′30″N, 25°42′12″E
8	Laajav 2	5 × 100 m	*Picea (Pinus, Betula)*	12	13	10	124	136	7	62°15′31″N, 25°41′55″E
9	Laajav 3	30 × 35 m	*Picea (Pinus, Betula)*	62	80	15	416	457	14	62°15′33″N, 25°41′59″E
10	Laajav 4	35 × 40 m	*Picea (Pinus)*	60	30	15	115	122	10	62°15′23″N, 25°41′57″E
11	Laajav 5	30 × 40 m	*Picea, Betula (Pinus)*	59	30		26	67		62°15′28″N, 25°42′40″E
12	Könk 1	15 × 100 m	*Picea*	100	70	15	236	941	64	62°15′01″N, 25°39′43″E
13	Könk 2	25 × 40 m	*Betula (Pinus)*	70			7			62°14′49″N, 25°39′48″E
14	Kanav	20 × 150 m	*Betula, Pinus (Picea)*	110	90	20	56	53	5	62°13′46″N, 25°54′52″E
15	Petäjäv	25 × 35 m	*Picea, Pinus*	10			34			62°19′49″N, 25°25′07″E
17	Yl. Kotaj	25 × 25 m	*Picea (Pinus, Betula)*		30	10		177	31	62°13′03″N, 25°37′00″E
18	Hanhij	20 × 25 m	*Picea (Betula, Pinus)*		30	14		129	8	62°13′57″N, 25°37′24″E
19	Keur 1	25 × 35 m	*Picea (Sorbus, Betula)*			20			20	62°13′37″N, 25°42′12″E
20	Kuusim 1	20 × 20 m	*Picea, Pinus (Betula)*			7			7	62°12′44″N, 25°29′47″E
21	Kuusim 2	20 × 20 m	*Picea (Betula, Pinus)*			7			9	62°12′51″N, 25°29′41″E
22	Kuusim 3	25 × 40 m	*Picea (Betula, Pinus)*			7			7	62°13′04″N, 25°29′40″E
23	Kuusim 4	30 × 40 m	*Picea, Pinus, Betula*			10			27	62°13′17″N, 25°29′54″E
24	Sip 3	15 × 70 m	*Betula, Picea (Alnus)*		40	15		190	23	62°12′41″N, 25°44′10″E
25	Laajav 6	30 × 70 m	*Pinus, Betula (Populus)*		40			36		62°15′37″N, 25°42′08″E
26	Muur 1	20 × 30 m	*Pinus (Betula, Picea)*		26			30		62°06′39″N, 25°39′40″E
27	Muur 2	25 × 30 m	*Betula (Sorbus, Picea)*		28			56		62°06′44″N, 25°40′05″E
28	Salama	25 × 40 m	*Picea, Betula*		40			164		60°48′35″N, 25°39′24″E
29	Tukki	15 × 25 m	*Betula (Pinus, Picea)*		31			48		60°49′08″N, 25°38′38″E
30	Villähde	15 × 25 m	*Picea (Pinus)*		30			107		60°55′07″N, 25°44′28″E
31	Viljan	30 × 40 m	*Picea, Betula, Populus*		30			117		60°49′53″N, 25°35′37″E
32	Pennala	20 × 50 m	*Picea, Pinus, Betula*		40			107		60°54′53″N, 25°41′39″E
33	Kaup	30 × 30 m	*Picea*		30			50		60°51′58″N, 25°33′40″E
34	Puhd	10 × 30 m	*Picea, Pinus*		30			14		63°42′18″N, 27°25′10″E
35	Pyör	15 × 25 m	*Picea (Betula)*		27			26		63°38′25″N, 27°28′04″E
36	Piha 1	20 × 30 m	*Picea, Betula (Pinus)*		25			15		63°41′41″N, 27°30′44″E
37	Piha 2	25 × 40 m	*Picea, Betula, Pinus*		30			14		63°41′42″N, 27°30′43″E
41	Must 1	20 × 30 m	*Picea (Populus, Betula)*			21			16	62°12′51″N, 25°41′22″E
42	Must 2	20 × 45 m	*Picea*			15			46	62°12′54″N, 25°41′36″E
47	Leivo 3	30 × 50 m	*Betula, Pinus (Picea)*			20			6	61°52′19″N, 26°00′07″E
48	Leivo 4	25 × 40 m	*Picea, Betula (Pinus)*			20			7	61°52′09″N, 25°59′44″E