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HOST-INDUCED ASSORTATIVE MATING IN HOST RACES OF THE LARCH BUDMOTH

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Abstract.—The likelihood of sympatric speciation is enhanced when assortative mating is a by-product of adaptation to different habitats. Pleiotropy of this kind is recognized as important in parasites that use their hosts as a long-range cue for finding mates, but is generally assumed to have limited applicability for most other organisms. In the larch budmoth, *Zeiraphera diniana* (Lepidoptera: Tortricidae), sympatric host races feed on larch or pine. *Zeiraphera diniana* females attract males (call) by releasing host-independent long-range pheromones. Pheromone composition differs strongly between host races, but we show in an experimental field study that cross-attraction can occur at a rate of 0.03–0.38. Cross-attraction to larch females increases when they call from neighborhoods (8-m radius) rich in pine or from pine trees. Cross-attraction to pine females similarly increases when calling from neighborhoods rich in larch, but there is no significant effect of calling substrate. Males, as well as females, of this species preferentially alight on their own host, and in neighborhoods where their own host is common. This effect of tree species and host neighborhood on assortative mating is therefore due, at least in part, to the numbers of males of each host race present within approximately 200 m^2 surrounding the female. This proximity effect is enhanced by the clumped distributions of the hosts themselves. Host chemistry might also affect pheromone production and/or response directly, but we have evidence neither for nor against this. This work provides empirical evidence that host adaptation has a pleiotropic effect on assortative mating in a species with host-independent long-range mating signals. Sympatric speciation via pleiotropy between ecological traits and assortative mating may thus be more common than generally supposed: Clumped resource distributions and habitat choice by adults are widespread.

Key words.—Assortative mating, host races, pleiotropy, speciation, *Zeiraphera diniana*.

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Assortative mating is a key to speciation because it allows related forms with divergent ecological adaptations to coexist in sympatry (Kirkpatrick and Ravigné 2001). In populations under disruptive selection, recombination will break up associations between loci affecting survival and those determining mate choice, thus leading to a low probability of speciation (Felsenstein 1981). If, however, assortative mating is pleiotropically controlled by the same loci that are under disruptive selection, antagonism between recombination and selection is reduced so that initial assortative mating can then be enhanced by reinforcement, and speciation becomes much more likely (Slatkin 1982; Rice 1984; Rice and Hostert 1993; Kelly and Noor 1996; Futuyma 1998; Dieckmann and Doebeli 1999; Kirkpatrick 2000; Servedio 2000; Kirkpatrick and Ravigné 2001).

In theory, speciation can also be produced even in the absence of pleiotropy (Kondrashov 1986; Kondrashov et al. 1998; Dieckmann and Doebeli 1999), but it is as yet unclear whether the assumptions of these models are met in nature, and it remains true that pleiotropy would make sympatric speciation a great deal easier. The most celebrated case of sympatric speciation, in the apple maggot (*Rhagoletis pomonella*), involves strong pleiotropy between host choice and assortative mating (Feder et al. 1994). In this and several other likely cases (*Eurosta solidaginis*, Craig et al. 1993, *Enchenopa* treehoppers, Wood and Keese 1990), the degree of assortative mating is directly determined by habitat or host choice.

However, pleiotropy between habitat choice and mate choice has been considered unlikely in most other organisms, in which mating behavior does not depend directly on cues from the habitat (Futuyma 1998; Tregenza and Butlin 1999). In many plant-feeding insects mate finding is relatively independent of host cues. For example, in most Lepidoptera, females call for males using long-range pheromones, and the host plant is not required for mating. Plant chemistry can directly influence production and release of pheromones in Lepidoptera (McNeil and Delisle 1989; Landolt and Phillips 1997), but these effects are weak and not known to cause assortative mating. To our knowledge, no previous studies have investigated whether host plant adaptation affects assortative mating in the Lepidoptera. Mating behavior in most moths can be divided into two stages. In the first stage, males follow plumes of pheromone released by distant calling females. Although the male may select pheromone plumes to follow, the female cannot choose at this stage. After males have encountered a female, a second, short-range stage of mating behavior potentially involves both male and female choice.

In this paper, we investigate the first stage of mating, long-range pheromonal attraction, in the larch budmoth, *Zeiraphera diniana* (Lepidoptera: Tortricidae). Two host races of Tortricidae studied here feed on larch (*Larix decidua*) or cembran pine (*Pinus cembra*). *Zeiraphera diniana* is renowned for regular outbreaks, which cause conspicuous defoliation of larch forests in the Alps. Population density on larch oscillates in an eight- to 10-year cycle; at their peak, populations may be 10^5 times denser than during a crash. What triggers a population outbreak is still not fully understood, but causes of population crashes likely involve delayed effects of defoliation on the quality of larch foliage in subsequent years (Baltensweiler 1993). Populations on pine appear more stable (Baltensweiler et al. 1977), although few data exist.

Although morphologically indistinguishable as adults, larch and pine races differ genetically at a number of traits,

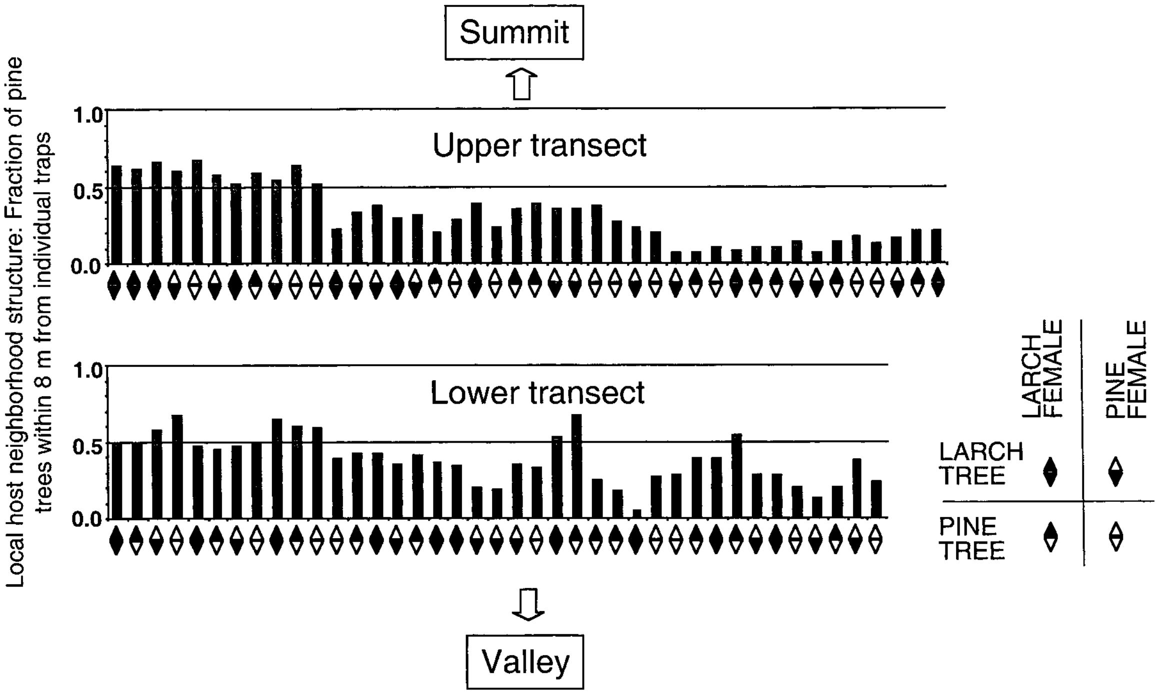

FIG. 1. Schema of the cross-attraction experiment with live females. The diagram shows the four types of traps (larch females on larch trees, larch females on pine trees, pine females on larch trees, and pine females on pine trees) and their order along the upper and lower transects, as well as fraction of pine trees within an 8-m radius of each trap.

including larval color pattern and allele frequency at the allozymes *Mdh-s, Idh-s,* and *Pgm* (Emelianov et al. 1995). Eggs are laid preferentially on the appropriate host (Bovey and Maksymov 1959). Females of each race produce a blend of two pheromone components (Baltensweiler et al. 1978), but in opposite ratios. Larch-race females produce virtually 100% trans-(E)11-tetradecenyl acetate (E11–14:Ac), with variable traces of trans-(E)9-dodecenyl acetate (E9–12:Ac). Pine-race females, in contrast, produce pheromone blends of 1:1000 E11–4:Ac to E9–12:Ac. Males respond maximally to pheromone blends of their own race (Priesner and Baltensweiler 1987a). As in other moths (Roelofs and Carde 1977; Roelofs et al. 1987; Löfstedt 1993), pheromone blends (Guerin et al. 1984) and male response (Roelofs et al. 1971; Baltensweiler et al. 1978; Priesner 1979; Priesner and Baltensweiler 1987a,b) in *Z. diniana* are independent of the host on which larvae are reared. However, although these differences between the host races are often strong, none are completely diagnostic, and given that flight periods overlap, there is plenty of opportunity for gene exchange. In addition to intermediacy for host race morphological and genetic traits, *Z. diniana* males with intermediate male electroantennogram response have been found in natural populations (Priesner and Baltensweiler 1987a).

Our experiments were designed to answer the following questions: Is long-range pheromone attraction assortative? Does the calling substrate (the host tree from which females call) affect assortative attraction? Does the host neighborhood structure affect assortative attraction? Do host races differ significantly in diurnal timing of pheromone communication, and does this contribute to assortative attraction?

MATERIALS AND METHODS

Study Site and Collections

Experiments were performed during summer 1997 on the south-facing slope of Val Bever (46°26′N, 9°50′E, elevation 1800 m), a side-branch of the Upper Engadine Valley, eastern Switzerland. The site consists of larch forest with a variable admixture of cembran pine. Larvae were collected from larch and pine in Val Bever in 1997, the year of the experiment, and reared to adulthood. Forty individuals of each race were analyzed electrophoretically to determine local allele frequencies. In addition, live females of each host race were used in the long-range attraction experiments described below.

Pheromone Cross-Attraction and Diurnal Timing

A total of 39 larch and 42 pine virgin females were used to lure wild males. Each female was placed singly in a 30-cm^3 metal mesh cage and was regularly supplied with drinking water and 5% sucrose solution. A twig of larch or pine was placed in each cage. These cages were placed individually into standard delta pheromone traps furnished with sticky cardboard inserts to trap males. Traps were positioned on larch or pine trees 15–20 m apart, 1.5–2.0 m above the ground, along two 700-m transects aligned in parallel across the slope and separated by approximately 40 m (Fig. 1). All four combinations of host race and calling substrate were used, each with approximately 20 females: (1) larch females caged with larch twigs in traps positioned on larch trees (LL); (2) larch females with pine twigs on pine trees (LP); (3) pine females with larch twigs on larch trees (PL); (4) pine females with pine twigs on pine trees (PP). Larch and pine trees over 4 m in height within an 8-m radius around each trap were counted, and the fraction of pine trees was used as an index of host neighborhood structure (Fig. 1). The four treatments were randomly distributed along the transects. Sticky inserts were checked, and trapped males were counted at 16:00, 20:00 and 08:00 h daily from 18 to 26 August. On 25 and 26 August, traps were additionally checked at 01:00. Every morning, inserts together with moths were removed from the traps and replaced with fresh inserts; the trapped males were frozen in liquid nitrogen and shipped to the laboratory for allozyme-based host race identification. Four traps not containing females were also positioned along the transects; none of these trapped any moths, so these negative controls are not mentioned further.

Host Race Identification Using Semidiagnostic Allozyme Markers

Cellulose acetate allozyme electrophoresis of trapped males was performed for two unlinked autosomal loci (*Mdh-s* and *Pgm*) and a sex-linked locus (*Idh-s*) according to methods given in Emelianov et al. (1995). In this study, no significant differences at *Pgm* were found between the host races, so there is little need to use this locus in host race identification. We use *Pgm* here because this locus is significantly differentiated in other years and sites (Emelianov et al. 1995); however, the results were not significantly affected by basing identification only on the remaining two loci. There are no fixed allozyme differences between the host races, so any three-locus genotype may with finite likelihood belong to either host race. However, allozyme frequency differences allow males of each host race to be identified with a high degree of accuracy. Given Hardy-Weinberg and linkage equilibrium within host races, as observed in field samples (Emelianov et al. 1995), the expected frequency of each three-locus genotype in larch and pine populations can be predicted using allelic frequencies from the reared larvae. The host race of each individual genotype was determined as the race in which the expected frequency of the genotype is highest (i.e., the procedure identifies the maximum-likelihood host race). The frequency of the same genotype in the less likely race is the error of identification for this genotype. The summed probability of incorrect host race identification for males estimated from these frequencies is 0.0010 for the larch race and 0.0039 for the pine race, an order of magnitude lower than the cross-attraction rates observed here.

Permutation Test for Correlation between Tree Data and Males Attracted

We were interested in testing for correlation between an independent variable (host neighborhood composition) and two dependent variables (numbers of larch and pine males attracted) for each trap-tree combination. Each trap cannot strictly be used as an independent sample, however, because of the possibility of spatial autocorrelation. Neighboring trapping sites will be independent neither in terms of forest composition, which may have a characteristic spatial scale of fluctuation greater than the distance between traps (clearly visible in Fig. 1), nor in terms of moth catches, because the characteristic spatial scale of male moth density, determined by dispersal, may also exceed the distance between neighboring traps. The standard *Z*-transformation of the correlation coefficient may therefore give inflated significance levels. Instead, we used a permutation test (similar to a bootstrap) to randomize the dependent variables relative to the independent variable while keeping the spatial structure of the data as constant as possible. The two transects were treated together as a 2 × 42 matrix, with each of 42 columns representing a pair of traps, one on each transect. The lower transect in fact consists of only 39 traps (Fig. 1), which were given label numbers 2–40. To make the data more manageable for permutation, three trapping locations were added to the lower transect: one (label 1) before the start of the transect, and two (41 and 42) after the end. The tree data, which are highly spatially autocorrelated (Fig. 1), were assumed to be for trap site 1 the same as the adjacent site 2, and for sites 41 and 42 to be the same as site 40. In contrast, the female/tree types and numbers of males caught in these sites were left missing for the purpose of permutation (i.e., no new trapping data was created). This treatment of missing sites is justified as follows: The permutation test is necessary because of spatial autocorrelation of trees; if the male attraction data are also autocorrelated, permutations would give correlation coefficients similar or greater than those obtained from the actual data more often than if sites were independent; the assumption of complete correlation of tree data between missing and adjacent sites, if anything, will inflate the autocorrelation estimate, and, therefore, is conservative. Product-moment correlation coefficients calculated from the actual data were compared with those obtained from data permuted while preserving spatial relationships. The dependent variables (males trapped, female type, and calling substrate) were permuted relative to the independent variable (the tree data, which was not changed) as follows: (1) male data exchanged between transects (vertical flip); (2) male data inverted along the transects (horizontal flip); and (3) male data moved laterally along the transects by a variable number of columns (0–41), with data running off the end of the transect being added in sequence to the other end. This gives a total of 2 × 2 × 42 = 168 possible permutations, that is, 167 plus the actual observations.

Trapping with Synthetic Pheromones

Additional delta traps baited with either larch pheromone (100:1 E11–14:Ac to E9–12:Ac) or pine pheromone (a 1:100 of the same compounds) were positioned on larch and pine trees well away from the main transect. Two main treatments (five traps each) were performed: larch pheromone traps on larch trees and pine pheromone traps on pine trees. In addition, five traps baited with pine pheromone were placed on larch trees. The sticky inserts were checked and trapped males were counted at 08:00, 16:00, and 20:00 h daily from 18 to 26 August. We did not identify the host race of attracted males using allozymes because of the very large numbers caught and because inferences made from results with synthetic pheromones do not depend strongly on host race identification.

TABLE 1. Allele frequencies in 1997 larval samples from Val Bever. Designation of loci and alleles as in Emelianov et al. (1995); *n*, number of analyzed genomes.

Locus	Allele	Frequency on larch	Frequency on pine
Idh-s		$n = 78$	$n = 82$
	0.65	0.410	0.024
	2.40	0.590	0.976
Mdh-s		$n = 84$	$n = 96$
	1.00	0.833	0.010
	5.50	0.167	0.990
Pgm		$n = 86$	$n = 96$
	0.87, 1.00	0.791	0.854
	1.12, 1.28	0.209	0.146

RESULTS

Allele Frequencies in the Larval Populations

Allele frequencies at *Mdh-s*, *Idh-s*, and *Pgm* from samples of larvae collected in 1997 at Val Bever are shown in Table 1. Allele frequencies on larch and pine differed weakly but significantly from those sampled in France and Switzerland in 1994 (Emelianov et al. 1995). Allele frequencies obtained from the same generation and site were therefore most appropriate for the likelihood-based identification of host races below.

Effect of Calling Substrate

Larch females calling from pine attracted significantly fewer males of their own race ($G_1 = 25.38$, $P < 0.001$) and males in general ($G_1 = 13.98$, $P < 0.001$) than larch females calling from larch (Table 2). Similarly, pine females on larch trees were attracted significantly fewer pine males ($G_1 = 6.70$, $P < 0.01$) and males in general ($G_1 = 8.62$, $P < 0.01$) than pine females on pine. The specificity of pheromone attraction by females positioned on their own hosts was high (Table 2). The probability of cross-attraction was only 0.033 for larch females on larch and 0.091 for pine females on pine. However, assortative pheromone attraction depended strongly on the host species from which the female called. The fraction of pine males among males attracted by larch females calling from pine was 0.377, significantly greater than on larch (0.033; $G_1 = 34.45$, $P < 0.001$). Cross-attraction by pine females calling from larch (0.063), however, was not significantly different from when they called from pine (0.091; $G_1 = 1.42$, $0.10 < P < 0.50$).

Effect of Host Neighborhood Structure

Circles of radius 8 m around each female contained on average 24.8 ± 1.08 trees. The estimated variance divided by the mean numbers of larch trees (s^2/m) was 5.6, whereas that for pine trees was 2.0. Both are greater than 1.0, the expectation for randomly (Poisson) distributed trees among trapping sites, indicating that both pine and larch trees are clumped. Clumped distributions could be due partly to some degree of mutual exclusion, so that a greater abundance of larch coincides with reduced abundance of pine and vice versa; a negative correlation between the local numbers of larch and pine trees around each trap ($r = -0.26$, $n = 81$, $P < 0.05$) provides some evidence for this.

There was a significant interaction between attractiveness of females and the host neighborhood (Fig. 2). Based on the permutation test, there was a significantly negative correlation between numbers of larch males attracted by larch females calling from larch trees and the proportion of pine in the neighborhood ($r = -0.65$, $P < 0.01$, Fig. 2A) and a less convincing negative correlation ($r = -0.35$, $P = 0.089$, Fig. 2B) for larch females calling from pine. Similarly, there was a significantly positive correlation between the neighborhood fraction of pine trees and the numbers of pine males attracted by pine females from pine trees ($r = 0.56$, $P = 0.012$, Fig. 2C) and from larch trees ($r = 0.70$, $P < 0.01$, Fig. 2D). There was no significant effect of host neighborhood on the numbers of males of the other host race cross-attracted (dashed lines in Fig. 2), but the power of this test is limited by low sample sizes. However, even assuming no effect on alien male attraction, it is clear that the significant own-male effect ensures the fraction of alien males decreases (and assortativeness of pheromone attraction increases) with the fraction of own host trees in the neighborhood (Fig. 2). A way of testing this overall hypothesis of assortative mating is to average absolute values of the correlation coefficients across all pairs and then performing the permutation test on the average correlation. Thus, the correlation coefficients averaged as follows: $r_{AV} = (-r_{LLxL} + r_{LLxP} - r_{LPxL} + r_{LPxP} - r_{PLxL} + r_{PLxP} - r_{PPxL} + r_{PPxP})/8$ (where, e.g., r_{PLxP} refers to the correlation coefficient for pine females, *P*, calling from larch trees, *L*, and the count of larch males, *xL*). The value for this overall correlation between attraction of own males and the fraction of own host in the neighborhood calculated from the data was $r_{AV} = 0.325$, greater than any obtained in any of the 167 permutations ($-0.276 \leq r_{AV} \leq 0.305$; $P < 0.010$).

TABLE 2. Attraction of larch and pine males by live females. Individuals were identified using three-locus allozyme genotypes. The probability of misidentification of individuals is negligible compared with the cross-attraction rates observed here; it is 0.0010 in males identified as belonging to the larch race and 0.0039 in males identified as pine race (see Materials and Methods). Therefore, among 658 males in the table, about two males identified as pine race will actually be larch race, and less than one male identified as larch race will actually be pine race.

Host race, host tree combinations	No. calling females	No. attracted larch males	No. attracted pine males	Cross-attraction of alien males	Total attracted males
Larch females on larch trees	19	118	4	0.033	122
Larch females on pine trees	20	33	20	0.377	53
Pine females on larch trees	22	12	184	0.063	196
Pine females on pine trees	20	26	261	0.091	287

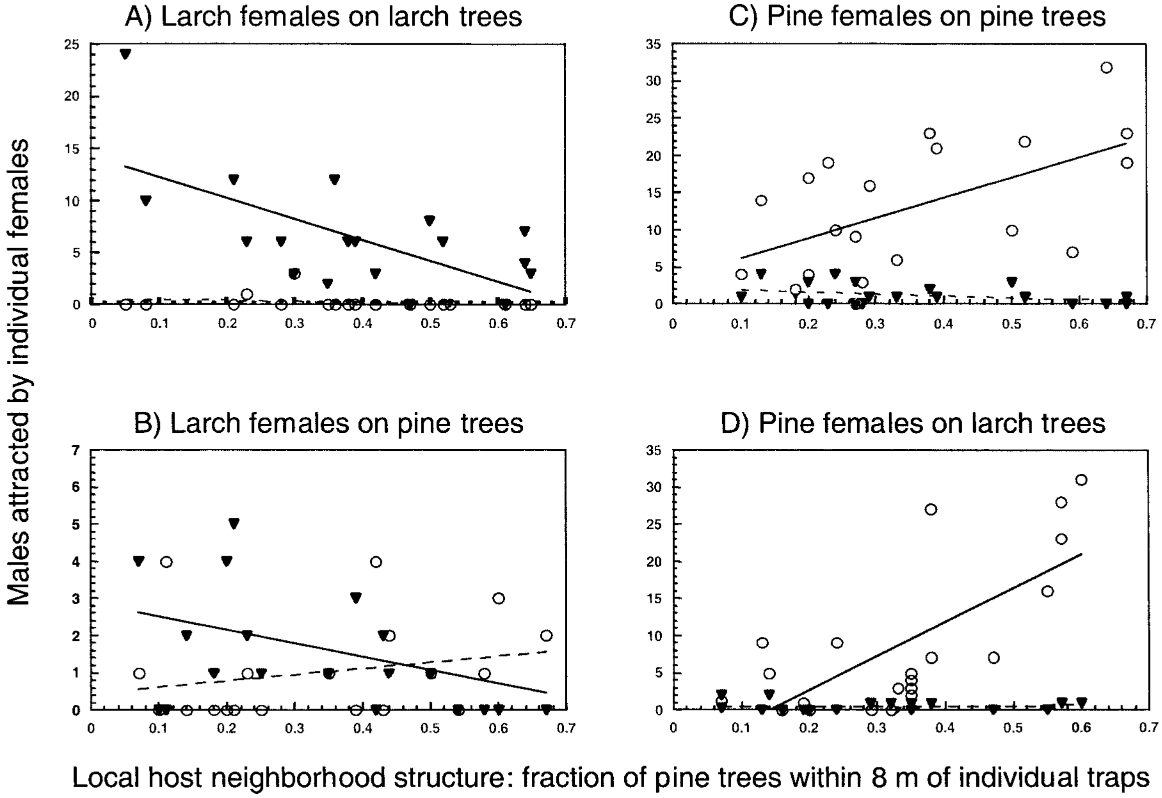

FIG. 2. Effect of host neighborhood on assortative attraction. Panels (A–D) represent the four types of traps, as in Figure 1. In each of these panels, the numbers of males of each race attracted by individual females are plotted against the fraction of pine trees in local host neighborhoods (i.e., 8-m radius circular patches of larch-pine forest surrounding individual traps). Larch male data are shown as solid triangles, and pine male data are shown as hollow circles. Solid regression lines represent males of the same race as the calling female, that is, larch males in panels (A) and (B) and pine males in panels (C) and (D). Similarly, dotted lines represent catches of alien males. Product-moment correlation coefficients (*r*) and statistical significance assessed by means of a permutation test are given in the text.

Diurnal Timing of Pheromone Attraction by Live Females

Pheromone activity in both host races of *Z. diniana* occurred mainly between 20:00 and 08:00 h (Fig. 3A), and male attraction peaked before 01:00 h (Fig. 3B). This peak of attraction appeared to be more prominent in the pine race where each female on average attracted 0.60 (0.44–0.76) males per night between 20:00 and 01:00 h and only 0.25 (0.13–0.38) males per night between 01:00 and 08:00 h (figures in parentheses are 95% confidence limits of the mean). Larch females showed similar, albeit statistically insignificant, differences between the two halves of the night; they attracted 0.23 (0.13–0.34) and 0.14 (0.06–0.21) males per female per night before and after 01:00 h, respectively. On 25 and 26 August, the larch females attracted a total of 35 males before 01:00 h and 21 males after 01:00 h; pine females attracted 96 and 40 males during the same periods. The temporal pattern of attraction did not differ significantly between host races ($G_1 = 1.18$, $0.10 < P < 0.5$).

Males Attracted by Synthetic Pheromones: Effect of Host and Diurnal Dynamics

Males from the two host races differed in their diurnal cycle of attraction to synthetic pheromone. Males were attracted to pine race pheromone almost equally by day (08:00–16:00) and by night. In contrast, larch pheromone was attractive to males almost exclusively at night (20:00–08:00). Traps baited with pine pheromone attracted on average 7.43 (5.73–9.15) males when positioned on pine trees and 5.33 (3.33–7.30) males per trap per night when positioned on larch trees ($P < 0.05$). Traps with larch pheromone on larch trees each attracted 4.35 (3.05–5.65) males per night.

DISCUSSION

Pleiotropy between Ecological Adaptation and Assortative Mating

Females calling from their own hosts cross-attract alien males with low probability (0.033–0.091). However, cross-attraction was significantly higher (0.377) for larch females calling from pine (Table 2). In addition, cross-attraction by both larch and pine females increased with the fraction of the alternative host in the immediate vicinity (Fig. 2). These effects of calling substrate and host neighborhood on assortative attraction might be explained in two ways. First, the results could be due to a proximity effect caused by the attraction of nearby males, whose specific host alighting preferences had previously caused them to alight in the vicinity of a calling female. The calling-substrate effect would then be due to attraction of males in the same tree as the calling

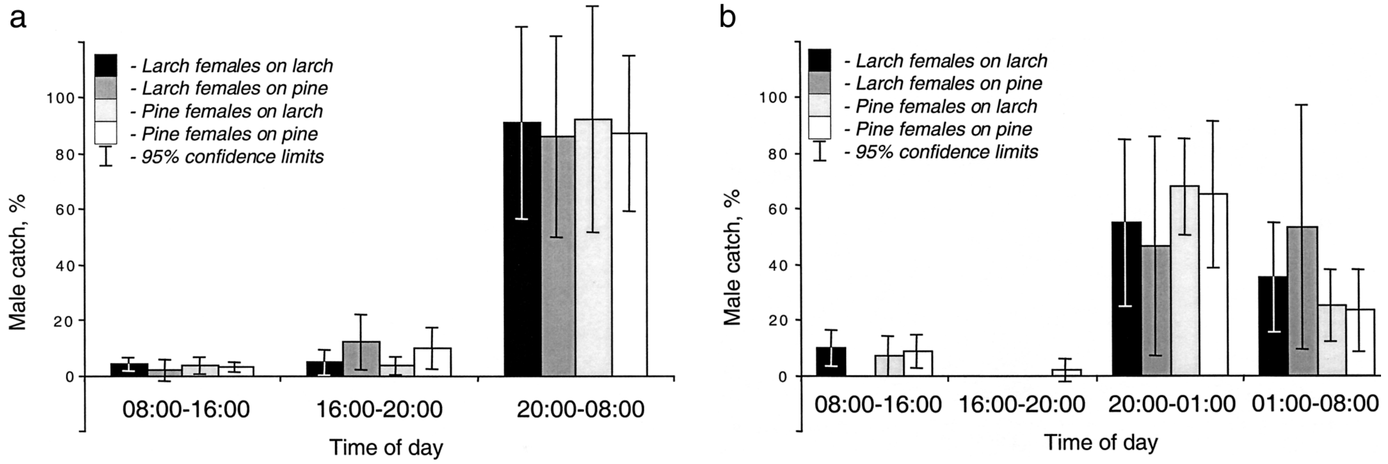

FIG. 3. Diurnal dynamics of pheromone communication. (A) Male attraction by live females during first and second halves of the day and during the night, 18–26 August. (B) Male attraction by live females during first and second halves of the day, and during first and second halves of night, 25 and 26 August only.

female, whereas the host-neighborhood effect would be due to attraction of males from trees of the same species nearby. Second, the interaction between host and assortative attraction could be a direct effect of host chemistry on pheromone production, pheromone release, and/or male response.

In the first explanation, males of the appropriate host race alight preferentially on their normal larval host (Emelianov et al. 2001), and thus concentrate near females calling from that host. The inverse correlation between the densities of larch and pine trees will amplify the effect of host preference on assortative attraction still further. Calling females also prefer to alight on their native host (Emelianov et al. 2001), which may cause a further concentration of males if they are preferentially attracted to higher concentrations of pheromone. In any case, it seems most likely that simple concentration of males around their own hosts is an important cause of the observed effect of host species on assortative mating. The importance of a potential direct effect of host chemistry on attraction cannot be ascertained without additional experiments. The amount of pheromone released has been shown to depend on host chemistry for a few other Lepidoptera (McNeil and Delisle 1989; Landolt and Phillips 1997) and cannot entirely be ruled out for *Z. diniana*. However, the composition of female sex-pheromone in Lepidoptera in general and *Z. diniana* in particular is independent of the host plant (see introduction).

Few doubt that habitat adaptation has a pleiotropic effect on assortative mating in parasites that use their host directly as a cue to find mates. For example, pleiotropy contributes strongly to reproductive isolation in host races of the apple maggot, *R. pomonella* (Feder et al. 1994), as well as to reproductive isolation in laboratory selection experiments on *Drosophila melanogaster* designed to imitate this situation (Rice and Salt 1990; Rice and Hostert 1993). Previously, it has not seemed plausible that habitat-induced assortative mating should evolve in organisms such as moths that find mates using long-range signals (Futuyma 1998; Tregenza and Butlin 1999). Because pheromone signals released from one host usually travel far enough to attract mates associated with other hosts, host-associated assortative mating would seem especially unlikely for night-flying moths: Female moths may attract males from as far as 4 km away (Gotz 1951). Contrary to these expectations, our experiments provide the first evidence for a host effect on assortative attraction in sympatric populations that use long-range sex pheromones. The effects we have demonstrated, together with evidence that both males and females choose to alight preferentially on their own host (Emelianov et al. 2001), clearly demonstrate a pleiotropic effect of host adaptation on assortative mating. Mate attraction in *Z. diniana* does not depend directly on the host as in *Rhagoletis*, but the pleiotropy we have found has the same effect and similarly may accelerate speciation (Rice 1984; Kondrashov 1986; Kondrashov et al. 1998; Dieckmann and Doebeli 1999; Kirkpatrick and Ravigné 2001). Our study joins a growing literature demonstrating pleiotropy between adaptation and assortative mating in a variety of other ecological contexts (Rundle et al. 2000; Jiggins et al. 2001; Podos 2001).

Asymmetry in Calling Specificity

Cross-attraction by larch females on pine was significantly greater than cross-attraction by larch females on larch. In contrast, cross-attraction by pine females did not depend on calling substrate (Table 2). A similar asymmetric effect of the host neighborhood presumably due to asymmetric cross-attraction was observed in an earlier study using synthetic pheromones in pure stands of each tree species. Traps with synthetic pine lures attracted only 81 males when exposed in a pure larch forest but 1071 males in a pure pine forest. In the same experiment, traps baited with larch lures attracted 3478 males in a larch forest and 2021 males in a pine forest (Baltensweiler 1998). The males were not identified to host race in that study, but we can assume that the extra males attracted to larch lures in the pine forest consisted largely of pine race. As in our case, pine males seemed to be more readily attracted to artificially released larch pheromone than were larch males to pine pheromone. Thus, the asymmetry in calling specificity must be due to difference in response behavior of larch and pine males rather than difference in calling behavior of females. Electroantennogram experiments

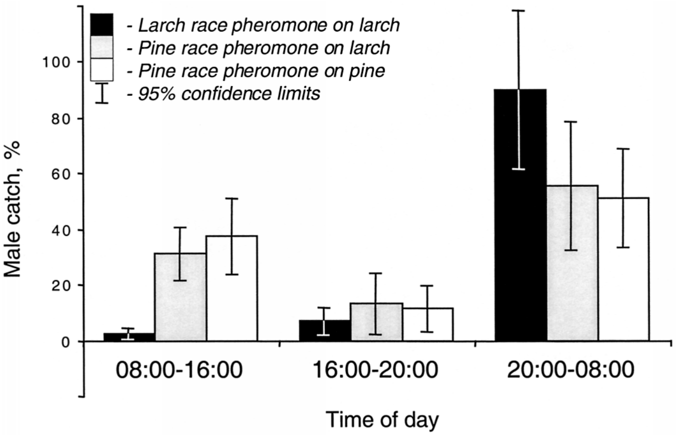

FIG. 4. Diurnal dynamics of male attraction by synthetic lures.

have shown that pine males respond to a wide range of pheromone blends (1:100, the normal pine female ratio, to less than 1:1 of E11–14:Ac to E9–12:Ac). Further increases in E11–14:Ac reduced but did not block the response of pine males. In contrast, even small admixtures of E9–12:Ac added to larch blends (normally > 100:1 of E11–14:Ac to E9–12:Ac) strongly reduced their attractiveness to larch males (Baltensweiler and Priesner 1988). Pine males therefore have a broader response window to pheromone blends than larch males. This could lead to greater cross-attraction by larch females than by pine females, as observed here.

The Potential for Hybridization between Host Races

We here show that assortative attraction is strongly affected both by the tree species used as a calling substrate and by the host neighborhood structure, but not by differences in the timing of pheromone communication. Mismatch between host trees and the host race of the calling female results in a significant increase in cross-attraction. Provided that assortative attraction results in assortative mating, the probability of hybridization will be strongly affected by host choice. Assortative mating at close quarters is weak: 28% of matings in laboratory choice tests were between host races (Drès 2001); so the occasional tendency for females to alight on and call from the wrong host or host neighborhood will be the major factor causing hybridization. By combining the probability that both males and females alight on their own hosts (0.79–0.89), estimates of assortative attraction obtained here (0.62–0.97) and of assortative mating at close quarters (0.70–0.80), the overall rate of hybridization in sympatry can be estimated as 0.02–0.04 (Emelianov et al. 2001).

The diurnal cycle of pheromone communication between males and calling females is very similar in the two races (Fig. 3), although more males were attracted to synthetic pine pheromone than to synthetic larch pheromone during the day (Fig. 4). Assuming that pine males were mainly attracted to pine pheromones in the absence of host-race identification, this suggests that pine males are active earlier than larch males; alternatively, the results may represent an artifact of using artificial lures, which contain pheromone blends in nonbiological concentrations. Additional work on this problem would be interesting, but our results highlight the problem of making strong inferences about moth behavior from work with synthetic lures. For the moment it seems clear that, if there are differences in the activity patterns of males, they have little effect on assortative mating, because there is no significant difference in the times of attraction to actual calling females (Fig. 3).

The host effects on assortative mating in *Z. diniana* are at least partly and perhaps mainly due to the density of male moths tracking the density of their host. The strong year-to-year fluctuation in the overall population density of the larch race could lead to temporal variation in assortative mating. When the density on larch is high, it is likely that an increased fraction of pine females would mate with the abundant larch males. Similarly, a crash in density on larch may lead to an increasing tendency for rare larch females to be mated by the now more abundant males of the pine race. Thus, the direction of gene flow could depend on the phase of the larch cycle. Periodic introgression of genes for pale larval color from the pine race into the larch race, whose mature larvae are normally black, may explain an observed synchrony between the population cycles and pale phenotypes on larch (Baltensweiler 1993). We are currently investigating evidence for gene flow and its periodicity using additional molecular markers.

Evolution of Host Races and Speciation

The host races studied here already have highly divergent pheromone systems that account for the majority of the assortative attraction we have measured, but this cannot have been the case initially. We here present alternative hypotheses for the origin of assortative mating now found in *Z. diniana*.

First, it is possible that the pheromone communication system diverged initially due to variation of pheromone blend and response; geographic pheromone polymorphism is known in some other moths (Löfstedt 1993). Polymorphic equilibria for different blends and responses are likely to be unstable (Butlin and Trickett 1997) so that alternative pheromone systems could rapidly be attained in different subpopulations. The resultant reproductively isolated pheromone races would still be unable to coexist (Kondrashov et al. 1998) unless character displacement led to a host shift by one of them or host specialization by both (if both hosts were used ancestrally), and, eventually, to the current association between host choice and pheromone blend.

It is also possible that divergence was initiated by adaptation to different hosts. Polymorphism in host choice would have had a pleiotropic effect on mate choice, as found here. Pheromone divergence of host races could then occur by reinforcement of host choice divergence. Recent models show that reinforcement occurs if levels of initial gene flow between sympatric forms are less than the random value of 0.5 (Kelly and Noor 1996), and more readily if the assortment trait is under direct divergent selection (Dieckmann and Doebeli 1999; Kirkpatrick 2000; Kirkpatrick and Ravigné 2001). Both of these conditions are satisfied in the case of habitat-dependent assortative mating found here. The process initiated by adaptive divergence of incipient host races could be completed by pheromone blend evolution. Assortative mating could then be maintained and enhanced by sexual selection acting against rare pheromone blends, rare male pheromone preferences (Butlin and Trickett 1997), and rare male and female host alighting preferences within each race.

In reality, assortative mating and host adaptation probably coevolve. The existence of assortative mating allows further host adaptation, and host adaptation triggers further assortative mating. Once the process of host or pheromone race formation is started, progress toward divergence in other aspects of biology may be enhanced. This work, together with related work on pleiotropy between ecological adaptation and assortative mating, shows that speciation in the presence of some gene flow may be rather more likely than hitherto seemed possible.

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