Biological Journal of the Linnean Society (1996), *58:* 287–306. With 5 figures

The effect of ant association on the population genetics of the Australian butterfly *Jalmenus evagoras* (Lepidoptera: Lycaenidae)

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Received 7 April 1995, accepted for publication 18 August 1995

Populations of the myrmecophilous lycaenid *Jalmenus evagoras* Donovan were assessed for genetic structure at three hierarchical spatial scales: sites, geographically-defined subpopulations, and subpopulations defined by species of mutualistic ant-associate. Estimates of Wright's F_{ST} generated from multilocus electrophoretic data revealed low, though significant, amounts of genetic structure. Most structure was observed at the level of geographic subpopulations, suggesting that adult butterflies do not exhibit preferential mating and oviposition along the lines of ant associate. The genetic structure data, together with estimates of Nei's genetic distance (*D*) for pairwise site and subpopulation comparisons, suggest that *J. evagoras* populations are spatially and temporally dynamic. These patterns are considered in the context of extinction and recolonization models. The extreme patchiness of *J. evagoras* populations stems from the stringent requirements of both host plant and host ant, contributing to an extinction/recolonization process. We discuss the key parameters influencing genetic cohesion versus differentiation under an extinction/recolonization regime, including mode of butterfly dispersal, site turnover rate, and the effects of host dispersal and phenology. This system provides a model of population-level consequences of certain mutualistic interactions as well as of a class of patterns arising from an extinction/recolonization process.

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ADDITIONAL KEY WORDS: — *Iridomyrmex* – Formicidae – genetic structure – extinction – recolonization – dispersal – Wright's *F*-statistics – mutualism – myrmecophily.

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INTRODUCTION

The Lycaenidae comprise almost a quarter of the approximately 17000 species of butterflies known, and the larvae of at least half of the species whose life histories have been described associate with ants (Pierce, 1987; Shields, 1989; Fiedler, 1991). These interactions range from parasitism to mutualism. In parasitic relationships, many of which are species-specific, larvae are usually carried by ants into the brood chamber of the ant nest where they prey upon the ant brood (Thomas *et al.*, 1989; Cottrell, 1984). In mutualistic interactions, larvae typically possess specialized glands that secrete ant appeasements and food in the form of sugars and amino acids (Malicky, 1969, 1970; Maschwitz, Wüst & Schurian, 1975). In return, ants tend and protect the larvae against parasitoids and predators. Some of these associations are obligate for the lycaenid, predation and parasitism being so intense that larvae require an attendant ant guard for survival (Pierce *et al.*, 1987). The great majority, however, appear to be facultative, with larvae only sometimes found with ants, often of varying species (e.g. Pierce & Easteal, 1986; Fiedler, 1991; Wagner, 1993).

Populations of lycaenid butterflies that rely upon protection by different species of ants provide a model system for the investigation of how obligate mutualistic interactions can affect the population structure of the participants. The influence of ecological variation on population genetic structure has been particularly well documented in plant/herbivore systems, especially among herbivores that feed on different hosts (e.g. Guttman, Wood & Karlin, 1981; Wood & Guttman, 1983; Thompson, 1988a,b; Barker, 1992). However, with the exception of studies of mutualist-mediated dispersal (i.e. pollinators, Campbell & Dooley, 1992; Fenster, 1991; and seed dispersers in plants, Furnier *et al.*, 1987; Tomback & Linhart, 1990), research into mutualisms has focused little attention on patterns of population structure and genetic differentiation.

Behavioural changes in host/habitat preference can have a rapid and dramatic impact on the population dynamics of a species. Many studies have correlated intraspecific variation in host use with population genetic differentiation (see Futuyma & Peterson, 1985; Thompson, 1988b), and several theoretical and empirical studies support the hypothesis that reduced gene flow between 'preference populations' can result in the formation of host races (see many examples in Otte & Endler, 1989). *Jalmenus*, whose ten species all have larvae that associate with ants, is representative of a number of Australian lycaenid genera whose species appear to have differentiated along ant rather than host plant lines. For example, at one of our study areas in Kogan, Queensland, three out of the ten species in the genus *Jalmenus, J. evagoras, J. ictinus* and *J. daemeli*, occur sympatrically and feed on the same species of host plant, *Acacia harpophylla* (brigalow). Adults of all three species can be seen flying at the same time from one host plant to another, but settling and laying eggs only on those plants populated by their respective species of attendant ant.

While the larvae of many species of *Jalmenus* appear to predominantly associate with only a single ant species throughout their ranges, *J. evagoras* is tended by several species of ants. At our main study area near Armidale, New South Wales, colonies of *J. evagoras* commonly associate with two ants, *Iridomyrmex anceps* and *I. vicinus*. At a given site, however, a colony is usually found with only one ant species, and these local associations persist from year to year.

A number of features of the life histories of myrmecophilous lycaenids indicate that mutualism with ants could have a strong impact on population structure. For

example, the extreme patchiness in the distribution of *J. evagoras* is a product of its dependency upon attendant ant colonies, resulting in a mosaic of overlapping requirements for both ant and host plant species (Smiley, Atsatt & Pierce, 1988; also see Jordano *et al.*, 1992). This produces considerable site fidelity: at one field site, 74 out of 80 marked individuals were observed almost daily for their entire estimated lifespans (about 3 days for females and 7 days for males; Elgar & Pierce, 1988). With their stringent habitat requirement of an overlap in the distributions of both appropriate host plants and suitable ant associates, obligately myrmecophilous lycaenids such as *J. evagoras* may be more susceptible to population subdivision than their non-ant-associated counterparts.

Moreover, the extremely patchy spatial distribution of ant-tended lycaenids may have influenced the mating systems of these insects, insofar as females in aggregated colonies are predictably located and relatively easy to monopolize. In *J. evagoras*, for example, males emerge before females (protandry) and regularly patrol trees containing conspecific pupae, their prospective mates. When a pupa is about to eclose, as many as 30 males may gather around it, forming a 'mating ball'. Copulation takes place upon eclosion, often before the teneral female has expanded her wings. Females mate only once, whereas males, if they are successful, have been observed to mate as many as seven times during their lifetimes (Elgar & Pierce, 1988).

Preliminary studies have revealed significant ant effects on butterfly size (Nested ANOVA, $F_{[1,495]} = 26$, $P < 0.01$) and larval aggregation ($F_{[1,225]} = 17.6$, $P < 0.01$). In colonies tended by *I. anceps*, male and female forewing lengths measured 21.14 ± 1.24 mm and 22.85 ± 1.87 mm respectively (means $\pm$ SD), whereas for colonies tended by *I. vicinus*, males and females averaged 20.65 ± 1.51 and 21.84 ± 2.08 respectively. Moreover, clusters of pupae tended by *I. anceps* contained on average twice as many pupae per cluster than clusters of pupae tended by *I. vicinus* (12.21 ± 20.47 versus 6.12 ± 6.72; means $\pm$ SD). The morphological and behavioural variation in *J. evagoras* subpopulations associating with different ant species underscores the potential role of ant association in influencing fitness and population-genetic parameters. Taken together, these factors suggest that the great diversity of the Lycaenidae may in part be a product of their specialized life histories, especially their association with ants.

An enormous amount of empirical and theoretical work has been devoted to the study of population differentiation and its potential role underlying the speciation process. Accordingly, much has been learned about the population genetic effects of drift, gene flow, and selection, and the many and varied ways in which these evolutionary processes jointly promote or undermine genetic cohesion of populations (e.g. Otte & Endler, 1989 and references therein). Wright (e.g. 1931, 1940, 1951, 1977) emphasized how local genetic differences could give rise to population differentiation through mechanisms such as differential dispersal among static, patchy, subpopulations — his shifting-balance process. Slatkin (1977, 1985, 1987) expanded this view, arguing that a process of extinction and recolonization of patchy subpopulations can provide enough gene flow to prevent population differentiation under some conditions.

The obligate mutualistic relationship of *J. evagoras*, and the extremely patchy distribution stemming from this relationship, makes this species an ideal candidate for analysing how ant association may have influenced its population genetic structure, and what our empirical assessment of population genetic patterns, together

with known aspects of *J. evagoras* natural history and ecology, may tell us about the population biology of this lycaenid. Two sets of factors potentially influence the genetic characteristics of *J. evagoras* populations: (1) the availability and distribution of 'ant + plant' sites, and (2) natal ant-species associate. We sought to evaluate the importance of these factors in shaping the population genetics of *J. evagoras* through a set of population genetic analyses employing allozyme markers. The analyses consist of three parts; (1) assessment of genetic structure at several hierarchical scales, defined both spatially (geographically) and by ant associate; (2) assessment of genetic distance within and between butterfly subpopulations defined geographically and by ant associate; and (3) assessment of the per-locus distribution of genetic variation between ant associate subpopulations. Together, these analyses permit us to assess the impact of mutualism on *J. evagoras* population genetics, and particularly whether populations of *J. evagoras* form 'symbiont races' analogous to 'host plant races' found in certain herbivorous insects.

EXPERIMENTAL SYSTEM

Jalmenus evagoras occurs in discrete, highly localized populations widely distributed throughout eastern Australia (Common & Waterhouse, 1981). The gregarious larvae consume at least 23 different species of *Acacia* (Nash, 1989). Eggs are laid in clusters, and larvae pupate directly on the host plant. Both the larvae and pupae of this species are dependent upon several species of dolichoderine ants of the genus *Iridomyrmex* for survival, and, in return, they provide the ants with secretions rich in carbohydrates and amino acids (Kitching, 1983; Pierce *et al.*, 1987). Larvae reared on higher quality host plants attract a larger ant guard and have higher survivorship in the field than larvae reared on lower quality host plants, and females prefer to lay eggs on the higher quality hosts (Baylis & Pierce, 1991). Females use ants as cues in oviposition (Pierce & Elgar, 1985), and males seeking mates also respond to the presence or absence of ants (Elgar & Pierce, 1988). Mating occurs on the host plant.

Ants in the genus *Iridomyrmex* are dominant in all habitats within Australia (Greenslade, 1985). Due to a high number of species and species groups, wide geographical range and lack of suitable morphological characters, the systematics of the genus has remained problematical for many years (Greenslade, 1979; Taylor, 1987). Recently S. Shattuck has undertaken a revision of the group, and we have lodged our samples with him for further identification. The species names presented here are based on determinations by R. Taylor and J. Greenslade, and vouchers are housed at the Australian National Insect Collection.

METHODS

Specimen collection and electrophoretic loci

Field sites ranged from the outskirts of Armidale, NSW, to Dorrigo, NSW, in an area covering about 150 square kilometers. In this region, colonies of *J. evagoras* commonly associate with two species groups of *Iridomyrmex, I. anceps* species group (sp.

25, Australian National Insect Collection) and *I. vicinus*. Throughout the region, *J. evagoras* feeds predominantly on *Acacia filicifolia, A. melanoxylon*, and *A. decurrens*.

We collected approximately 60 butterflies from each of 15 sites between January and February 1993 (Fig. 1; Table 1). Each butterfly was scored for sex, forewing length, and wing wear before being frozen in the field in a portable cryogenic container of liquid nitrogen. Specimens were later transferred to an ultralow-temperature freezer in the laboratory and maintained at –70°C until electrophoresis.

Specimens were analysed with horizontal starch-gel electrophoresis using pH 6.0 amine-citrate (morpholine) (= C) and pH 6.5 tris-citrate (= 4) continuous buffer systems (see Murphy *et al.*, 1990). Tissue preparation and staining procedures followed those reported in Shoemaker, Costa & Ross (1992), developed for another lepidopteran species. Of 33 enzyme loci screened, six were monomorphic and six polymorphic at the 99% level. The polymorphic loci are: *Aat-1* (E.C. 2.6.1.1; C buffer), *Acoh-1* (E.C. 4.2.1.3; 4 buffer), *Ak* (E.C. 2.7.4.3; C buffer), *Gpi* (E.C. 5.3.1.9; 4 buffer), *Pgm-1* (E.C. 5.4.2.2; 4 buffer), and *Sod* (E.C. 1.15.1.1; 4 buffer). The mean number of alleles per locus is 5.5 (range = 4–12) and the mean effective number of alleles is 1.43 (range = 1.06–1.85).

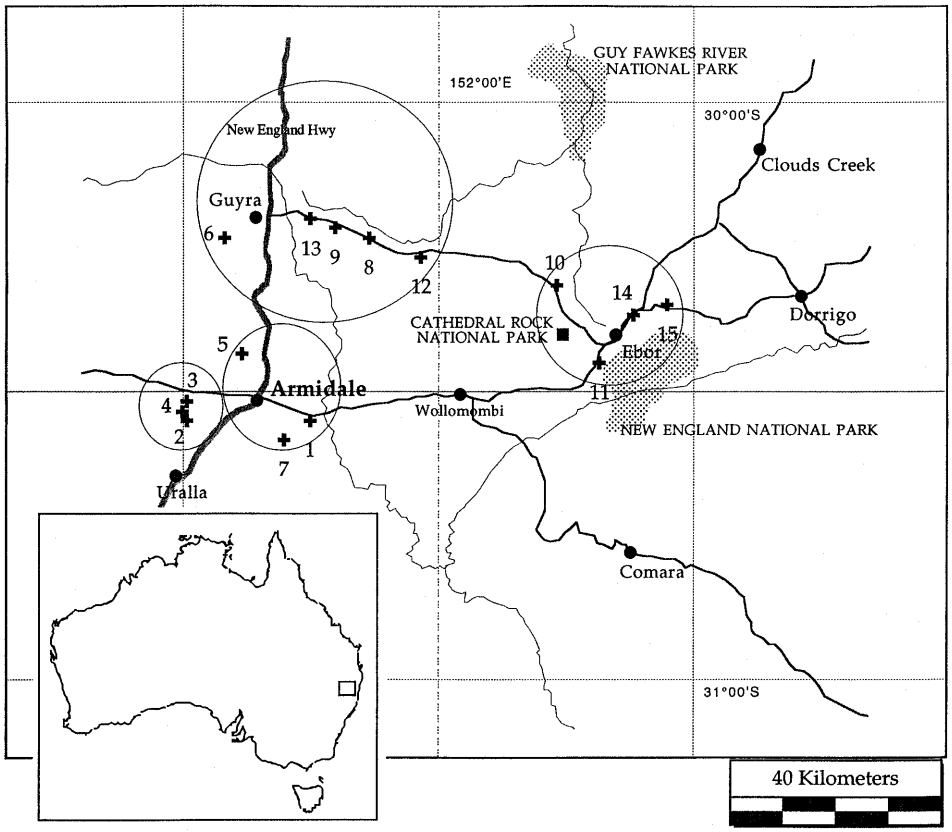

Figure 1. Map of *Jalmenus evagoras* sampling sites located in New South Wales, Australia. Butterflies at sites 1–7 are tended by *Iridomyrmex vicinus*; those at sites 8–15 are tended by *I. anceps*. The four geographically-defined subpopulations are circled.

TABLE 1. Summary of sample sizes, *Iridomyrmex* ant associate species, and host plants of the genus *Acacia* for 15 *Jalmenus evagoras* study sites sampled in 1993 in NSW, Australia (see Fig. 1).

Site				
No.	Name	*n*	Host ant	Host plant
1	Scary Rd.	61	*I. vicinus*	*A. filicifolia*
2	Kalinda Rd.	69	*I. vicinus*	*A. filicifolia*
3	Kendall Rd.	61	*I. vicinus*	*A. filicifolia*
4	Bilga Rd.	58	*I. vicinus*	*A. filicifolia*
5	New Holme	49	*I. vicinus*	*A. decurrens*
6	Elderberry	87	*I. vicinus*	*A. dealbata*
7	East Wood	61	*I. vicinus*	*A. filicifolia*
8	Brockley Stud	50	*I. anceps*	*A. dealbata*
9	East Brook	61	*I. anceps*	*A. filicifolia*
10	Spring Park	60	*I. anceps*	*A. filicifolia*
11	Cathedral Rk.	53	*I. anceps*	*A. melanoxylon*
12	Aberfoyle Turn.	62	*I. anceps*	*A. filicifolia*
13	Cutting	77	*I. anceps*	*A. dealbata*
14	Panton's Gully	66	*I. anceps*	*A. melanoxylon*
15	Bald Hills	66	*I. anceps*	*A. melanoxylon*

Population-genetic analyses

Genetic structure

Population genetic structure was assessed hierarchically using the procedure of Weir & Cockerham (1984) to estimate Wright's (1951) *F*-statistics in two sets of analyses (Fig. 2), herein termed 'Analysis 1' and 'Analysis 2'. Each analysis consisted of two hierarchical levels of population subdivision: Analysis 1 (Fig. 2A) treated sites nested within subpopulations, which were defined (1) geographically, and (2) by ant-associate species, while Analysis 2 (Fig. 2B) treated geographically-defined subpopulations nested within subpopulations delimited by ant-association. One site (no 6) in subpopulation 1 was eliminated from the latter analysis in order to completely nest geographically-defined subpopulations within ant-associate subpopulations (this site was characterized by *I. anceps* while all others in this subpopulation were characterized by *I. vicinus*; see Fig. 1).

Taken together, these related sets of analyses treat *J. evagoras* populations at three hierarchical spatial scales: sites, geographic subpopulations, and ant-associate subpopulations (Fig. 2C). This multi-level analysis is necessary to dissect the confounding effects of spatial separation and ant association on the partitioning of genetic variation in *J. evagoras* populations. These effects are confounded as a result of extreme population patchiness; *J. evagoras* requires the coincidence of host plant and ant associate (Smiley *et al.*, 1987), and the two ant associate species considered in this study infrequently occur together, in part due to competition and territorial behavior (e.g. Greenslade, 1987; Andersen & Patel, 1994). Thus, despite a lack of ant-site interdigitation, this hierarchical analysis partitions the *F*-statistics into contributions from each level of the hierarchy, making it possible to compute degrees of differentiation across levels.

The *F*-statistics, or inbreeding coefficients, were developed by Wright (1951) as hierarchically-related descriptors of the distribution of genetic variation in populations. The total inbreeding coefficient (F_{IT}) is decomposed into contributions from individual inbreeding (F_{IS}) and the inbreeding-like effects of population genetic

structure (F_{ST}) (Wright, 1951). We are especially interested in F_{ST}, also called the fixation index or standardized allele frequency variance, as an index of genetically effective movement patterns in the population.

The procedure of Weir & Cockerham (1984) for estimating *F*-statistics corrects lower-level genetic structure for the effects of higher-level structure, thereby providing unbiased estimates of the *F*-statistics at both levels. A jackknife procedure was employed to obtain estimates of the mean and standard error (SE) for all *F*-statistics following the method of Weir and Cockerham (1984). The jackknife SE estimation was performed in two ways: (1) jackknifing over the alleles of each locus independently (yielding the SE among alleles at single loci) and (2) jackknifing over all of the loci (yielding the SE among loci). The 95% confidence intervals were obtained from the standard errors by assuming the *t* distribution, and these were used to judge whether the mean values differed significantly from zero.

Genetic distance

Nei's (1978, 1987) genetic distance (*D*) was calculated for all pairwise sites and subpopulations using the program of Sattler & Hilburn (1985); our estimates of *D* are maximum estimates due to the exclusion of completely monomorphic loci from this

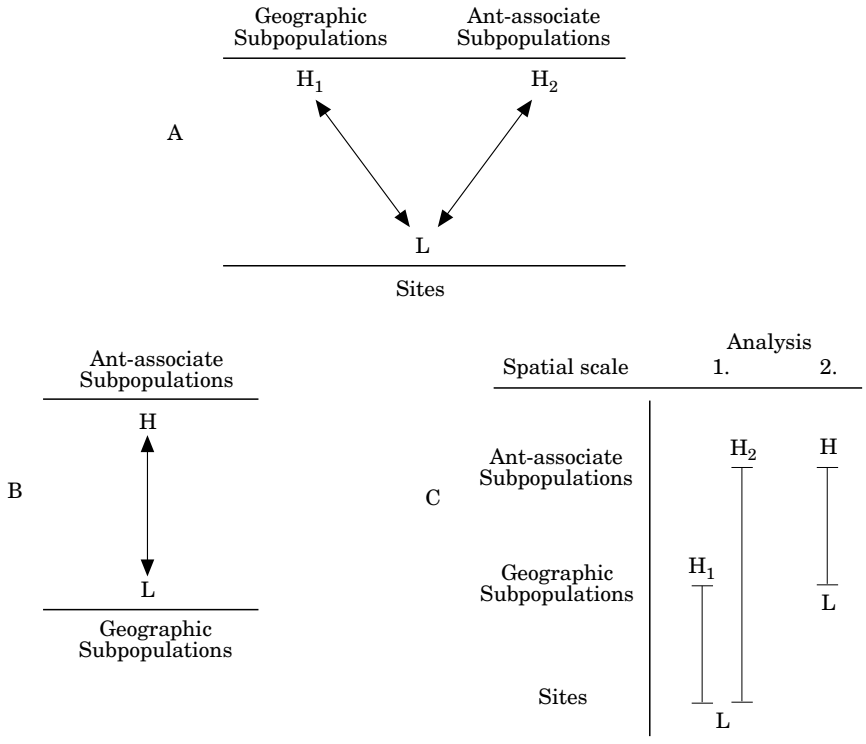

Figure 2. Schematic diagram of two *F*-statistics analyses. A, Analysis 1 consists of two sub-analyses differing in the way the higher spatial level of the population (H) is defined in the estimation procedure; the lower population level (L) is held constant in the two sub-analyses. H_1 = geographically-defined subpopulations; H_2 = ant-associate-defined subpopulations. B, Analysis 2 treats geographic subpopulations as the lower spatial level (L) by nesting completely within ant-associate subpopulations (H); this is accomplished by eliminating a single confounding site (site 6; Fig. 1). C, Summary of Analyses 1 & 2; taken together, these analyses permit partitioning of genetic variance at three hierarchical spatial scales: sites, geographic subpopulations, and ant-associate subpopulations.

study. Subpopulation-level allele frequencies were used to obtain distance values between geographically-defined and ant-defined subpopulations, respectively. Two tests of distance-partitioning were conducted, both employing the bootstrap resampling procedure of Weir (1990) to identify the degree and direction of difference in distance values. The first test considered paired sites within and between geographic subpopulations, and the second considered paired sites within and between ant-associate subpopulations. In each test, paired-site D values within each subpopulation were randomly resampled with replacement 1000 times. These values were then ordered to obtain the 95% confidence interval about the mean, permitting direct comparison of distance value distributions between and within the different subpopulations (Weir, 1990).

Allele-frequency distributions

The bootstrap resampling procedure of Weir (1990) was also employed to compare allele frequencies between ant-associated subpopulations of *J. evagoras*. In this analysis, the array of genotypes for each locus in each ant-associate subpopulation was reconstructed by random resampling 1000 times; the allele frequencies calculated for each of the reconstructed genotype arrays were then ordered to obtain the 95% confidence intervals about the mean frequencies. This technique allows, again, direct comparison of allele frequencies between the subpopulations of interest.

RESULTS

Genetic structure

Single locus and summary estimates of Wright's *F*-statistics for Analyses 1 and 2 are summarized in Tables 2 and 3, respectively. In Analysis 1, the lower level of the population (site) is held constant while the higher level (subpopulation) is alternately defined by simple geographic distribution of sites (Table 2, part I) or by ant-associate characterizing the sites (Table 2, part II). The small differences in site estimates across these two sub-analyses stems in part from differences in the sample-size correction factors in the estimation procedure (Weir & Cockerham, 1984). These correction factors are based on site and subpopulation number and sample size; thus, differences at otherwise identical spatial scales represent variation in the estimators and are not in themselves biologically significant.

Nearly all estimates of Wright's *F*-statistics are significantly non-zero in this population of *J. evagoras*. The modest magnitude of the estimates indicates low levels of inbreeding and weak genetic structure. Note that the total inbreeding (F_{IT}) in Analysis 1 is partitioned differently between the two sub-analyses: when the higher population level is defined geographically, most of the total inbreeding is explained by genetic structuring (F_{ST}) rather than by individual inbreeding (F_{IS}). Just the reverse is evident when the higher level is defined by ant-associate, indicating that the modest genetic differentiation observed at higher spatial scales in *J. evagoras* populations comes primarily from geographic effects.

This observation is underscored by the results of Analysis 2 (Table 3), in which the lower level of the population corresponds to the geographic subpopulations of Analysis 1 and the upper level corresponds to the ant-associate subpopulations. More

TABLE 2. Analysis 1 estimates of Wright's *F*-statistics (means±SE) for two spatial scales in *J. evagoras* populations. Subpopulation spatial scales were defined geographically (I) and by ant species associate (II). Single-locus values were obtained by jackknifing over alleles. Summary values were obtained by jackknifing over loci. *n*=15 sites, four geographic subpopulations, and two ant associate subpopulations.

		Site		Subpopulation	
Locus	F_{IT}	F_{ST}	F_{IS}	F_{ST}	F_{IS}
I. Geography					
Aat-1	0.014±4.6E-03*	0.013±1.2E-07*	0.002±4.7E-03	0.005±1.9E-06*	0.009±4.6E-03
Acoh-1	0.129±1.2E-03*	0.060±3.2E-04*	0.073±4.0E-04*	0.040±1.4E-04*	0.092±9.4E-04*
AK	0.055±1.8E-02*	0.056±4.2E-05*	–0.001±1.8E-02	0.015±1.5E-04*	0.039±1.6E-02
Gpi	0.031±1.1E-03*	0.061±1.2E-04*	–0.032±2.0E-03*	0.014±2.0E-05*	0.017±1.1E-03*
Pgm-1	0.035±6.4E-04*	0.031±7.8E-05*	0.004±4.7E-04*	0.020±3.6E-05*	0.016±8.3E-04*
Sod	0.007±2.2E-05*	0.015±2.4E-06*	–0.008±1.0E-05*	–0.008±9.1E-07*	0.015±3.1E-05*
Summary	0.061±4.2E-04*	0.048±4.7E-05*	0.014±4.0E-04*	0.019±3.5E-05*	0.043±2.9E-04*
II. Ant species					
Aat-1	0.015±4.9E-03*	0.013±5.6E-06*	0.002±4.7E-03	0.005±8.3E-06*	0.010±4.6E-03*
Acoh-1	0.117±1.0E-03*	0.048±2.5E-04*	0.073±4.0E-04*	0.008±1.5E-04*	0.110±4.6E04*
AK	0.050±1.7E-02*	0.051±1.9E-05*	–0.001±1.8E-02	–0.004±1.1E-05*	0.054±1.8E-02*
Gpi	0.031±1.2E-03*	0.061±8.6E-05*	–0.032±2.0E-03*	0.007±5.3E-05*	0.024±1.1E-03*
Pgm-1	0.035±8.1E-04*	0.031±1.2E-04*	0.004±4.7E-04*	0.009±3.8E-05*	0.027±5.4E-04*
Sod	0.009±2.2E-05*	0.017±2.5E-06*	–0.008±1.0E-05*	0.001±6.0E-10*	0.009±2.2E-05*
Summary	0.057±3.7E-04*	0.044±4.0E-05*	0.014±4.0E-04*	0.007±1.1E-05*	0.051±3.5E-04*

*Values differ significantly from zero.

genetic structuring is found at the level of geographic subpopulations (F_{ST} = 0.031) and little is attributable to partitioning among ant-associate subpopulations (F_{ST} = 0.008). At the generally higher spatial scales of Analysis 2, the inbreeding-like effects of lower-level structure contribute significantly to total inbreeding (F_{IS} = 0.049 and 0.072 for geographic and ant-associate subpopulation levels, respectively). This is in contrast to the case of the lower spatial scale, that of sites, at which F_{IS} values are small (F_{IS} = 0.014 for both sub-analyses).

TABLE 3. Analysis 2 estimates of Wright's *F*-statistics (means±SE) for two spatial scales in *J. evagoras* populations. Subpopulations were defined geographically (lower-level) and by ant species associate (higher-level). Single-locus values were obtained by jackknifing over alleles. Summary values were obtained by jackknifing over loci. *n*=four geographic subpopulations, and two ant associate subpopulations.

		Geographic subpopulation		Ant-associate subpopulation	
Locus	F_{IT}	F_{ST}	F_{IS}	F_{ST}	F_{IS}
Aat-1	0.053±1.2E-03*	0.006±3.8E-06*	0.047±1.1E-03*	0.001±1.0E-05*	0.052±9.8E-04*
Acoh-1	0.107±1.5E-03*	0.052±8.6E-04*	0.058±2.0E-03*	0.013±7.3E-04*	0.096±1.1E-03*
AK	0.158±4.5E-04*	0.020±3.8E-05*	0.141±7.1E-04*	–0.020±6.9E-04*	0.174±2.5E-04*
Gpi	0.059±7.7E-04*	0.032±1.8E-05*	0.027±9.7E-04*	0.015±7.9E-05*	0.044±1.3E-03*
Pgm-1	0.055±2.4E-04*	0.026±5.3E-05*	0.030±1.4E-04*	0.007±1.0E-04*	0.049±7.3E-05*
Sod	0.009±4.5E-05*	–0.001±4.8E-08*	0.010±4.3E-05*	0.001±1.4E-07*	0.008±4.1E-05*
Summary	0.079±2.8E-04*	0.031±6.0E-05*	0.049±3.0E-04*	0.008±4.6E-05*	0.072±3.2E-04*

*Values differ significantly from zero.

TABLE 4. Nei's genetic distance (mean $D \pm$ SE) for *J. evagoras* populations defined geographically and by ant-associate.

Population pair	$D \pm$SE
Geographic subpopulations	
1,2*	0.0136±0.0084
1,3*	0.0065±0.0053
1,4	0.0055±0.0036
2,3	0.0034±0.0016
2,4*	0.0089±0.0047
3,4*	0.0056±0.0034
Ant-associate subpopulations	
1,2	0.0045±0.0023

*Geographic comparisons across ant species

Genetic distance

The average genetic distance between sites ($D = 0.11 \pm 0.008$ [SD]) as well as between higher-level subpopulations (Table 4) is generally low; this is consistent with the modest degree of genetic structure inferred from estimates of the *F*-statistics. Moreover, these low values are maximum estimates because completely monomorphic loci were excluded from the estimation procedure. Variation in genetic distance among sites and among subpopulations was sufficient to warrant further study of their distribution, however. This was done with the bootstrap resampling procedure of Weir (1990). The results, including the bootstrap mean *D* values and their 95% confidence intervals (CI), are graphed in Figures 3 and 4.

Comparing *D* values within and between higher-level subpopulations, no clear relationship exists between genetic distance and either spatial separation or ant association. Of the six pairwise geographic comparisons (Fig. 3), three paired subpopulations [(1,2), (1,4), & (2,4)] exhibit distance patterns expected under a simple isolation-by-distance model: low within-subpopulation *D* relative to between-subpopulation *D*. Two subpopulation-pairs [(2,3) & (3,4)] exhibit the patterns counter to those expected: *lower* between-subpopulation *D* than both within-subpopulation values. The remaining pair, (1,3), exhibits an intermediate pattern where *D* is much higher in one of the subpopulations than the other, and the between-subpopulation *D* lies between the two within-subpopulation values.

The distribution of distance values between vs. within ant-associate populations (Fig. 4) reveals a tendency to greater distances between sites found with different ant species. In other words, the *greatest* distances are found across ant-associates. Moreover, the 95% CI from populations associating with only one of the ant species overlaps with that of the between-species values, while the other lies completely outside the CI of the between-species values.

Allele-frequency distributions

A total of 22 of 34 allele comparisons exhibit frequency differences between the two ant-associate subpopulations (Table 5). Eleven of these comparisons involve alleles present at a frequency of 0.01 or lower in at least one of the two

subpopulations and are accordingly relatively uninformative. The distribution of genetic variation in *J. evagoras* populations is highly variable: disjunct allele frequencies are not consistent across all loci (neglecting the rare alleles at *Ak* and *Sod*), nor are they consistent across all alleles at a given locus.

DISCUSSION

This study provides a detailed picture of the spatial distribution of genetic

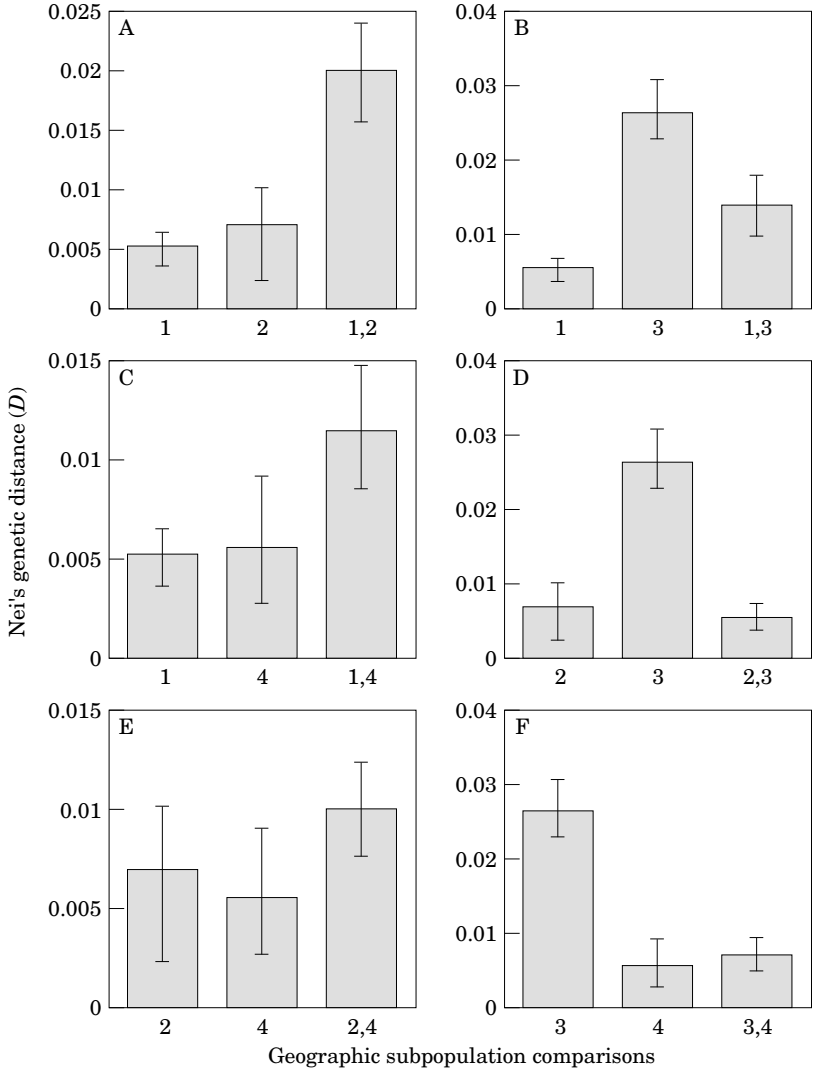

Figure 3. Comparisons of mean genetic distances (*D*) and their 95% CIs within and between geographic subpopulations, generated with 1000 bootstrap resampling iterations following the procedure of Weir (1990). A, B, E & F represent subpopulation comparisons across ant species associate, C & D represent subpopulation comparisons within ant species associate. Note that only comparisons A, C, & E conform to isolation-by-distance expectations.

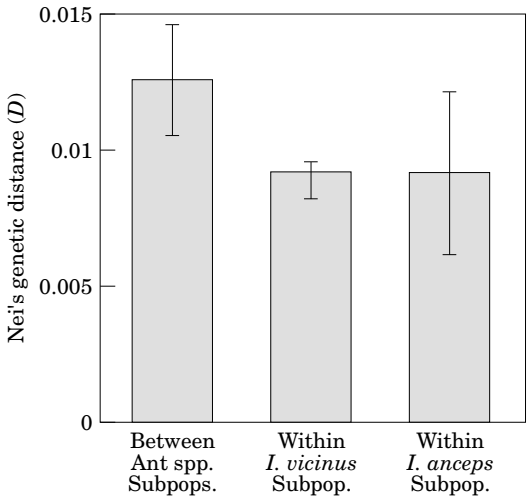

Figure 4. Comparisons of mean genetic distances (*D*) and their 95% CIs among sites within and between ant-associate subpopulations, generated with 1000 bootstrap resampling iterations following the procedure of Weir (1990). While mean genetic distance among sites between ant associate subpopulations is greater than those within both subpopulations, the CIs indicate there is no consistent pattern of differentiation.

variation in *J. evagoras* in this region. This distribution reflects gene flow, the levels of which are inferred from the F_{ST} values to be generally high in the population. A trend exists toward increased genetic similarity among populations with increased spatial scale (differentiation among sites > among geographic subpopulations > among ant associate subpopulations). In other words, genetic exchange is somewhat attenuated among localized sites, but as these sites are grouped into successively larger subpopulations equilibrium conditions are approached. For a given total inbreeding level, the ratio of F_{ST} to F_{IS} will often vary at different spatial scales. In our study population, this ratio decreases as spatial scale increases. Sites exhibit the greatest degree of genetic differentiation, and the population viscosity observed at this level is responsible for relatively higher measures of inbreeding found at higher levels.

Of particular interest with respect to the hypothesis of 'host-ant race' formation is the partitioning of genetic variation at higher levels of the population (geographic subpopulation and ant-associate subpopulation). Comparing F_{ST} values across these levels, it is evident that little structure is attributable to ant association, while most structure is partitioned along geographic lines (Tables 2 & 3). These results suggest that ant association is a poor predictor of genetic partitioning in this population. In other words, there appears to be no evidence that *J. evagoras* adults systematically select oviposition sites or mates on the basis of natal ant association.

If the *F*-statistics lead us to reject a model of ant-based gene flow reduction, the genetic distance and allele frequency distributions lead us to reject the alternative models of panmixis or simple isolation-by-distance. Although the low genetic distance values generally underscore the patterns inferred from the *F*-statistics, the pairwise distance estimation procedure, which provides distance values for each site pair and population pair, permits a more detailed view of spatial relationships than do the *F*-statistics. The distribution of these values indicates that movement patterns

of this species are not a simple function of geographic separation. A plot of genetic distance against geographic distance reveals a weak positive relationship (Fig. 5), but the regression slope does not differ significantly from zero ($t = 1.813$, $0.1 > P > 0.05$; Zar, 1984). Similarly, the allele frequency distributions further indicate that there is no simple divergence pattern between subpopulations. The bootstrap-generated allele frequencies show variable degrees of differentiation (Table 5); while all loci possess some alleles exhibiting disjunct frequencies, all alleles for a given locus do not exhibit such patterns. Such a frequency distribution may arise with restricted site flow between the populations, under which conditions the allele frequencies are subject to drift such that some become disjunct while others remain similar. The totality of evidence, however, including the *F*-statistics and genetic distances, suggests that these distributions arose not as a consequence of gene-flow

TABLE 5. Bootstrap mean allele frequencies and 95% CIs for six polymorphic allozyme loci from *J. evagoras* populations associating with two ant species. Values were generated with 1000 resampling iterations (Weir, 1990). Boxes indicate significant differences in mean allele frequency between the ant associate populations based on the overlap of 95% CIs.

Allele	*I. vicinus* Associates	*I. anceps* Associates
Aat-1^1	0.983 (0.974–0.991)	0.962 (0.948–0.974)
Aat-1^2	0.001 (0.000–0.003)	0.014 (0.006–0.023)
Aat-1^3	0.016 (0.009–0.025)	0.021 (0.013–0.030)
Aat-1^4	0.000	0.001 (0.000–0.003)
Acoh-1^1	0.729 (0.699–0.759)	0.700 (0.667–0.731)
Acoh-1^2	0.129 (0.107–0.151)	0.048 (0.032–0.065)
Acoh-1^3	0.087 (0.067–0.108)	0.178 (0.151–0.205)
Acoh-1^4	0.000	0.010 (0.003–0.018)
Acoh-1^5	0.055 (0.039–0.071)	0.065 (0.049–0.082)
Ak^1	0.907 (0.886–0.927)	0.883 (0.860–0.904)
Ak^2	0.037 (0.026–0.050)	0.023 (0.014–0.034)
AK^3	0.055 (0.040–0.071)	0.090 (0.073–0.111)
Ak^4	0.000	0.003 (0.000–0.008)
Gpi^1	0.752 (0.724–0.778)	0.683 (0.654–0.712)
Gpi^2	0.010 (0.004–0.018)	0.001 (0.000–0.003)
Gpi^3	0.048 (0.035–0.061)	0.033 (0.022–0.046)
GPi^4	0.000	0.016 (0.009–0.025)
Gpi^5	0.001 (0.000–0.003)	0.000
Gpi^6	0.092 (0.074–0.110)	0.114 (0.094–0.135)
Gpi^7	0.000	0.052 (0.038–0.068)
Gpi^8	0.086 (0.068–0.104)	0.023 (0.013–0.033)
Gpi^9	0.003 (0.000–0.008)	0.014 (0.007–0.022)
Gpi^{10}	0.007 (0.002–0.012)	0.062 (0.047–0.079)
Gpi^{11}	0.000	0.002 (0.000–0.005)
Gpi^{12}	0.000	0.000
Pgm-^{11}	0.867 (0.845–0.890)	0.901 (0.881–0.921)
Pgm-^{12}	0.050 (0.036–0.065)	0.015 (0.007–0.024)
Pgm-^{13}	0.047 (0.033–0.062)	0.003 (0.000–0.008)
Pgm-^{14}	0.036 (0.024–0.050)	0.077 (0.060–0.095)
Pgm-^{15}	0.000	0.003 (0.000–0.008)
Sod^1	0.871 (0.850–0.892)	0.844 (0.819–0.870)
Sod^2	0.124 (0.103–0.146)	0.153 (0.129–0.178)
Sod^3	0.005 (0.001–0.009)	0.000
Sod^4	0.000	0.002 (0.000–0.006)

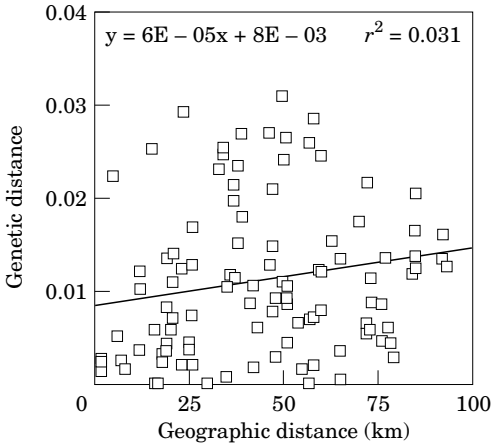

Figure 5. Regression of Nei's genetic distance (*D*) for site pairs against geographic distance. The slope of the regression line is not statistically different from zero ($0.1 > P > 0.05$; $t = 1.813$), leading us to reject a model of simple isolation-by-distance.

restriction by host ant selection, but rather from the stochastic effects of a fluid and patchily distributed population.

Our data suggest that myrmecophily influences the population biology of *J. evagoras* through the subtle dynamic of shifting site distribution: rather than serving as a template underlying assortative mating on the part of the butterflies, the obligate relationship with *Iridomyrmex* ants contributes to the marked physical mosaicism of colonies of *J. evagoras*, which in turn sets the stage of a type of population dynamics generally not found in continuously-distributed species. This pattern is consistent with those reported for many other butterfly species, some of which experience severe habitat fragmentation as a result of narrow hostplant requirements (e.g. Collins & Thomas, 1991; Dennis, 1992). Insofar as *J. evagoras* colonies are spatio-temporally dynamic, the population-genetic patterns we observe may be best understood in the context of extinction and recolonization of acceptable patches.

The physical patchiness of *J. evagoras* populations arises as a result of the dual requirement of host plants and ant mutualists (Pierce *et al.*, 1987). Moreover, the patchiness of the host plant and host ant populations themselves further contributes to the creation of an extreme environmental mosaic for *J. evagoras* (Smiley *et al.*, 1988), a mosaic that is likely to favour repeated extinction and recolonization events as new sites form and existing sites become unacceptable. We have evidence that appropriate conditions for *J. evagoras* are often limited under field conditions. We frequently observe larvae consume the foliage of their host plant and then starve as a consequence. Larvae often migrate from their host plant when it is defoliated, but their chances of finding new hosts with appropriate tending ants are low.

The mosaic of appropriate host plant/ant sites is not only spatial; it is also temporal as a consequence of the phenology and demographics of both hosts. Some species of tending ants, like *I. anceps*, preferentially patrol only small (= young) *Acacia* trees, and *J. evagoras* selects such trees as oviposition sites (Smiley *et al.*, 1988); as trees in the range of *I. anceps* age, they become increasingly unacceptable to the ants and therefore to the butterflies as well. Site acceptability is also governed by ant colony and host plant lifespan. Many *Acacia* species are rapidly growing and short-lived

colonizers of fire-disturbed habitat, including most of the species supporting *J. evagoras* in our study population (Tame, 1992). Host plant life history characteristics such as growth rate and life span thus ensure that sites acceptable to *J. evagoras* are ephemeral. Less is known about the demographics of the ant species involved. Colonies of *I. anceps* are monogynous, and queens have survived for up to 5 years in the laboratory.

Population genetic effects of extinction/recolonization

Slatkin (1977) first pointed out that processes of extinction and recolonization may under some conditions constrain population differentiation, in contrast to Wright's (1940) view that these processes are expected to enhance differentiation rates. The conditions favouring one or the other pattern concern (1) the source of colonists, (2) relative size of colonizing group, (3) the frequency of 'background' migration relative to colonization, and (4) frequency of local extinction (Slatkin, 1977, 1985, 1987).

The first condition, colonist source, is of primary importance. Mode of colonization may be conceived as a continuum, with colonists originating from a single randomly-determined source population on one end of the continuum ('propagule pool' model), to colonists comprising several source populations at the other end of the continuum ('migrant pool' model) (Slatkin & Wade, 1978; Wade, 1982; Slatkin, 1985, 1987). The difference between these colonization patterns concerns how much of the genetic variation of the population is sampled by founder groups. Slatkin (1977) found that a propagule pool colonization pattern more readily lends itself to population differentiation than a migrant pool pattern. Factors such as time to site extinction and effective population size are also key parameters influencing the likelihood of differentiation (Slatkin, 1985, 1987), though other authors argue that the number of colonists founding new populations (K) relative to twice the number of 'background' migrants moving between existing populations ($2Nm$) is of greater importance (Wade & McCauley, 1988).

Extinction/recolonization in Jalmenus evagoras *populations*

The data presented here for *J. evagoras* relate to models of extinction/recolonization through a consideration of (1) the mode of colonizing new sites, and (2) the rate of colonizing new sites.

(1) *Colonist origin.* Most populations strictly conform to neither migrant-pool nor propagule-pool dispersal models, lying somewhere between these extremes according to various spatial and temporal stochastic factors (Wade & McCauley, 1988). *J. evagoras* dispersal behaviour lies closer to the migrant-pool end of the spectrum. This inference is based on the observation that sites often support relatively large numbers of butterflies; our data indicate that inbreeding is quite low at these sites (Tables 1 & 2), making it unlikely that the many butterflies commonly observed at sites are descendants of a single foundress. Furthermore, *J. evagoras* is highly efficient at locating available ant + plant sites, making it unlikely that only a single individual locates sites as they become available (Smiley *et al.*, 1988).

(2) *Rate of colonization relative to background migration*. Wade & McCauley (1988) draw an important distinction between colonization (K) and migration (Nm). The difference between these alternatives hinges on whether individuals are arriving at new, vacant sites, or at sites currently supporting other members of the population. Sites empty upon arrival are thus colonized, while occupied sites merely receive additional migrants. This distinction is important because if gene flow is solely of the migration form, the population is more consistent with a classical, static model of population structure and not subject to the unique dynamic of extinction/recolonization.

The relative magnitude of colonization vs. migration clearly depends on several factors, among them the frequency of formation of new sites and site persistence. Our data indicate that *J. evagoras* is a relatively vagile species, with gene flow among sites occurring at moderately high rates as inferred from the *F*-statistics. How can we identify this gene flow as migration vs. colonization, *sensu* Wade & McCauley (1988)? Given that *J. evagoras* is generally bivoltine (Common & Waterhouse, 1981) and that sites are known to persist at least on the order of several years (N.E. Pierce, pers. obser.), much of the genetic movement is likely to be of the 'background migration' type. The potential of colonization, as distinct from migration, is contingent on the availability of *new* sites. Given the chance nature of site formation, which depends on both ant and plant dispersal and life-history, overall site turnover and colonization opportunities are also likely to be high (see below). Wade & McCauley (1988) argue that the extinction/recolonization process is most likely to lead to population differentiation under a regime of propagule-pool founding when K is small relative to $2Nm$. The fact that our study population is weakly differentiated and is inferred to approximate a migrant-pool model of founding is consistent with these theoretical expectations, and may also suggest that the colonization rate (K) among sites is quite high in our populations.

The process of genetic differentiation by drift is generally insensitive to extinction rate according to Wade & McCauley (1988), in contrast to the conclusion of Slatkin (1985, 1987) that drift can facilitate differentiation when the average time to extinction (in generations) is greater than N_e, the effective population size. It is worth noting in this vein that time to site extinction may well be greater than the effective population size of this species. Our population sample exhibits a highly skewed sex ratio (77.4% male; average M:F ratio among sites = 4.50, n = 941) as a result of strong protandry. Protandry and mate competition leads to a high variance in male mating success in *J. evagoras* (Elgar & Pierce, 1988) which is expected to reduce the effective population size (Hartl & Clark, 1989). If sites persist for several years and the butterflies have two or three generations per year (Common & Waterhouse, 1981), the average time to extinction is likely to be smaller than N_e, a situation that would also be consistent with our observation of weak population differentiation following Slatkin's (1987) formulation.

The importance of parameters such as time to extinction and effective size depends upon colonization mode. Whitlock & McCauley (1990) showed, for example, that the effect of extinction rate on within- and among-population genetic variance is increasingly important as colonization approaches a propagule-pool pattern. Slatkin (1987), Wade & McCauley (1988), and Whitlock & McCauley (1990) agree that migrant-pool dispersal in an extinction and recolonization process has the effect of preventing population differentiation by genetic drift; these authors differ primarily in their interpretation of which processes and conditions (e.g. colonization

rate, site longevity, effective population size) are of greatest importance in effecting or preventing differentiation. *J. evagoras* appears to exhibit population dynamics prohibiting differentiation, consistent with the conclusions of theoretical models. Our observations of modest genetic structure and inferred high rates of gene flow provide predictions for (1) predominant mode of colonization [should be closer to a migrant-pool pattern], (2) frequency of site formation [new sites should form with moderate to high frequency], and (3) time to site extinction [should be moderate to short].

Further studies of the population biology of *J. evagoras*, its host plants and associated ants will be separately necessary before the population dynamics and interactions between these species can be fully understood. In particular, with respect to the lycaenids, field tests focusing on the behavioural ecology of site colonization should be conducted, with the aim of learning the number and source of colonists as well as the rapidity of colonization and extinction. With respect to the ants and host plants, a rigorous assessment of site dynamics, focusing on spatial and temporal persistence, will help us to understand the ecological milieu influencing *J. evagoras* population genetic patterns. This empirical approach was fruitfully applied by McCauley (1989) in a study of patch extinction in the cerambycid beetle *Tetraopes tetraophthalmus*.

In the *Jalmenus evagoras* system, potential effects of the ants on host plant populations are particularly worthy of consideration. *Acacia* spp. are well-known myrmecochorous plants, the seeds of which are dispersed by foraging ants (Berg, 1975). Andersen & Ashton (1985) conducted a study of the rates of seed removal by ants in different ecological sites in southeastern Australia, and found that all *Acacia* seeds presented as bait were removed by foraging ants, among them several *Iridomyrmex* species. In general, *Iridomyrmex* spp. appear to be less important than other ant species (e.g. *Pheidole & Rhytidoponera* spp.) in seed removal (Hughes & Westoby, 1992), but it is clear that ant-mediated dispersal together with local fire history are important determinants of the spatial distribution and age structure of *Acacia* populations (L. Hughes, pers. comm.).

The modest degree and spatial partitioning of population genetic structure exhibited by *J. evagoras* populations indicates that assortative mating associated with host-ant specificity is unlikely to play a dominant role in shaping the population dynamics of this species. *J. evagoras* thus does not support a model of ant-association driven diversification of myrmecophilous lycaenids (Pierce, 1984). Ant-association is important in another respect, however: geographic isolation, and especially spatial and temporal patchiness imposed by ant specificity, appears to create an extinction/recolonization process in which the genetic cohesion of these populations is contingent upon patterns of dispersal and site availability. While this population displays a large degree of genetic cohesiveness at present, the strong environmental mosaic we observe at lower spatial scales may lend itself to reduced genetic exchange should certain key parameters change. For example, an increase in physical patchiness might shift the population further toward a propagule-pool colonization mode and permit greater opportunities for the action of drift. Thus, it is possible that situations might arise in which ant interactions may contribute significantly to processes promoting diversification among lycaenid butterflies, and this may have been the case historically. That such parameter changes may be easily induced by human disturbance underscores the fragility of the population ecology of this and many other butterfly species (Collins & Thomas, 1991) and the importance of recognizing population dynamics in conservation efforts.

ACKNOWLEDGEMENTS

We thank Andrew Berry for collecting specimens and for advice with the data analyses. Jeff Feder, Konrad Fiedler, Lesley Hughes, Roger Kitching, Marty Kreitman, and Ken Ross all offered useful advice, insights, and criticisms at various points during the study. Bill Piel kindly made Figure 1. The field collections in Australia would have been impossible without the hospitality of Bev and Roger Kitching, Sue McIntyre and John Lewis in Armidale, and Jim and Helen Skene in Ebor. We thank John Dugan from Ecosystem Management for allowing us to use facilities at the University of New England, and Jim Fittler, who greatly facilitated our work there. Nevil Amos, Bill Ballard, Joy Bergelson, Dave Brown, Jeff Mathews, David Nash, Leslie Schenkel, and Diane Wagner assisted in locating field sites and collecting butterflies. Finally, this manuscript benefited from the insightful comments of two anonymous reviewers.

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