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Ant predation as a selective agent on overwintering eggs in a field cricket mosaic hybrid zone

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Abstract

Hybrid zones may serve as arenas for the evolution of barriers to gene exchange while still allowing the exchange of favorable alleles between species. Differential introgression of linkage groups indicate favored, neutral, and disfavored genomic regions with respect to intrinsic (epistatic interactions, linkage to specific genes) and extrinsic (environmental selection, behavior, ecological interactions) environments. Additionally, the ability of alleles to introgress will depend on hybrid zone structure (opportunity for individuals to interact) and maintenance (criteria for favorable traits). Thus, trait differences that influence local distribution within these hybrid zones contribute to species coexistence and persistence in the face of hybridization. The mosaic hybrid zone between two North American field crickets, *Gryllus firmus* and *G. pennsylvanicus*, is structured by underlying soil heterogeneity in Connecticut. Despite this, it is not clear what role soil type plays in maintaining this hybrid zone. A companion study demonstrated that abiotic characteristics of different soil types do not cause significant viability selection on diapausing cricket eggs that overwinter in the soil. Instead, other environmental factors, such as winter weather, play larger roles in egg survival. Here I investigated one biotic factor that potentially has a large effect on egg survival — predation by ants. These results show that when ants are present, egg mortality is dramatic, increasing 2.5 fold against areas without ant infestations. Although not significant, there is some indication that ant activity may vary among sites and soils. Hence, this biotic factor may influence the distribution of cricket species in this hybrid zone and consequently the dynamics of their interactions.

Key words

mosaic hybrid zone, crickets, *Gryllus*, egg predation, viability selection, habitat segregation

Introduction

In nature, many hybrid zones represent the results of a natural selection "experiment" that has occurred for thousands of generations over the entire genomes of the hybridizing species. This experiment frequently involves large numbers of individuals (the interacting populations of each species), and it is replicated over time and space. Importantly, this experiment occurs in a natural, environmental background. The result of hybridization between two species may include the evolution of genetic barriers (Howard 1993), but hybridization also may lead to the exchange of favorable alleles between species (Rieseberg *et al.* 1999). Differential introgression of genetic linkage groups indicates favored, neutral, and disfavored genomic regions with respect to intrinsic (epistatic interactions, linkage to specific genes) and extrinsic (environmental selection, behavior, ecological interactions) environments (Barton & Gale 1993, Rieseberg *et al.* 1999). Additionally, the ability of alleles

to introgress will depend on hybrid zone structure (opportunity for individuals to interact) and maintenance (criteria for favorable traits) (Cain *et al.* 1999, Sadedin & Littlejohn 2003).

The structures of hybrid zones traditionally have been described as clinal in nature (Endler 1977, Barton & Hewitt 1985, Barton & Gale 1993). For many hybrid zones, this clinal structure was assumed to result from intrinsic selection against hybrid individuals and consequently to be independent of the environment influences (Bazykin 1969; Barton & Hewitt 1985, 1989; Barton & Gale 1993). It is now becoming increasingly clear that many hybrid zones are structured by habitat heterogeneity (Harrison & Rand 1989, Howard *et al.* 1993, Ross & Harrison 2002, Vines *et al.* 2003). Mosaic hybrid zones are those that are structured by a patchwork of habitats, where each species is found on alternate habitats, and hybridization between parental types occurs primarily at patch boundaries (Levins 1968; Slatkin 1973; Harrison 1986, 1990; Howard 1986; Harrison & Rand 1989; Ross & Harrison 2002). Thus, the maintenance of these hybrid zones likely has an extrinsic, environmental component (Harrison 1986, 1990; Arnold 1997, but see Searle 1993). However, the mechanism that drives habitat associations is not clear for many mosaic hybrid zones, and many hybrid zones may have multiple mechanisms of maintenance. Regardless of the mechanisms, these hybrid zones provide a superb opportunity to understand how habitat segregation can play a prominent role in structuring and maintaining hybrid zones, and thus create ecological and reproductive isolation.

The mosaic hybrid zone between the North American field crickets, *Gryllus firmus* (Scudder) and *G. pennsylvanicus* (Burmeister), is structured by the underlying patchwork of soil types (Rand & Harrison 1989, Harrison & Rand 1989, Ross & Harrison 2002). However, while the soil type mosaic determines the structure of this cricket hybrid zone, what maintains the cricket-soil association is less clear. A companion experiment (Ross & Harrison 2006) executed in conjunction with this study, suggested that while different soil types (loam *vs* sand) may induce different selection pressures on these cricket species, within each soil type, selection is the same for both cricket species. Further, other abiotic and biotic factors in the environment, such as winter weather, habitat-specific predators, and nonsoil habitat variables, may provide stronger selection pressures than soil itself (Ross & Harrison 2006). In this companion experiment, a subset of egg containers contained ants [*Lasius neoniger* (Formicinae) Emery] or evidence of ant activity (soil modification). Consequently, the opportunity arose to examine the effect of predation by ants on overwintering cricket eggs in various experimental

conditions. In this study, I investigate one potential selective agent in the context of the *Gryllus* mosaic hybrid zone: ant predation on cricket eggs.

The *Gryllus* hybrid zone

G. firmus, the "beach cricket", lives in coastal and lowland habitats along the North American eastern seaboard from Florida to Connecticut (Lutz 1908; Fulton 1952; Alexander 1957, 1968). It is found on sands and other soils with high sand content. *G. pennsylvanicus* occurs in inland and upland areas from northern Georgia, north along the Appalachian Mountains into Ontario and west to California, Oregon and Washington (Lutz 1908; Fulton 1952; Alexander 1957, 1968). *G. pennsylvanicus* is found on loam and other soils with loamy character, frequently in old fields or pastures. *G. firmus* is a larger, lighter colored cricket, and females have relatively long ovipositors compared with their body length. In contrast, *G. pennsylvanicus* is smaller and darker, and females have relatively short ovipositors.

These two field cricket species hybridize in a long, narrow zone that extends at least from North Carolina through Connecticut, approximately along the eastern edge of the Appalachian Mountains (Harrison & Arnold 1982, Harrison 1986, Fig. 1). Although the overall position of the hybrid zone on a regional scale may be influenced by climatic restrictions for each species, or by the recent history of range expansion, local hybrid zone structure is determined by the underlying habitat patchwork of different soil types (Rand & Harrison 1989, Ross & Harrison 2002).

In Connecticut, the field cricket hybrid zone has been described as mosaic in nature (Harrison 1986). Relatively pure parental types are found throughout the zone, and habitat heterogeneity (in soil type) allows these parental types to interact directly within the zone, forming local populations with bimodal distributions for many characters and rapid transitions in character frequencies through space (Harrison 1986, Ross & Harrison 2002). Few F_1 hybrids are found, although multigeneration backcross individuals occur, and the bimodal distribution of multilocus genotypes (Harrison & Bogdanowicz 1997) is typical for many hybrid zones (Jiggins & Mallet 2000). The mosaic nature of the hybrid zone has been demonstrated for morphological characters (Harrison 1986), allozymes (Harrison 1986), mtDNA (Harrison *et al.* 1987), and anonymous nuclear markers (Harrison & Bogdanowicz 1997). Within the hybrid zone, *G. pennsylvanicus*-like individuals and alleles are found on patches of loam soils, whereas *G. firmus*-like individuals and alleles are found on sandy soils (Rand & Harrison 1989, Harrison & Rand 1989). This genotype-soil type association is robust, even at very fine spatial scales (< 20m) across soil-patch boundaries (Ross & Harrison 2002).

Both *G. pennsylvanicus* and *G. firmus* have an intimate relationship with the soils on which they live. Throughout the hybrid zone, eggs of both species spend the winter in diapause in the soils in which they are laid. In Connecticut, most eggs are deposited in August and September and do not hatch until the following May. *G. firmus*, with a relatively long ovipositor, exhibits the presumptive adaptation necessary to deposit eggs deeply in well-draining sandy soils and therefore avoid desiccation (Masaki 1979, 1986). Likewise, the short ovipositor of *G. pennsylvanicus* may be better suited for more moist, loam soils, where shallow deposition of eggs enables newly hatched crickets to escape the dense soil matrix (Alexander & Thomas 1959; Masaki 1979, 1986; Bradford *et al.* 1993; Carrière & Roff 1995). During their time in the soil, eggs must take up water

as well as exchange oxygen and other gases with their environment (Masaki & Walker, 1987) while avoiding freezing, desiccation, and mechanical stresses due to the soil movement of freeze-thaw cycles. These factors are strongly influenced by the composition of the soil (*i.e.*, soil type; McKeague 1978) as well as local winter weather conditions, such as temperature, precipitation, and snow cover (Masaki 1979, 1986; Bradford *et al.* 1993; Mousseau & Roff 1995). Additionally, eggs are susceptible to various predators, including ants, spiders, and micro-organisms.

Although a clear association with soil type is found in natural populations, in the laboratory both species exhibit an oviposition preference for loam soils (Ross 2000). Therefore habitats imposing selection on crickets, rather than crickets selecting habitats, would appear to be the more likely explanation for the current distribution. As a consequence of the close relationship between crickets and soil in Connecticut and the proximity of the *G. firmus* populations to the limits of their species' distribution, selection directly on overwintering egg viability may play an important role during the egg stage of the life cycle (Carrière *et al.* 1997). If so, selection could be important in determining observed patterns of habitat segregation and in maintaining the hybrid zone between these two recently diverged taxa.

Materials and methods

Egg Collection.—Adult female crickets were collected during September 1996 from Connecticut populations (Fig. 1, Table 1). These (or nearby populations) have been characterized previously using genetic markers, morphological characters, or both (Harrison 1986, Harrison & Rand 1989, Harrison *et al.* 1987, Harrison & Bogdanowicz 1997). Crickets were transported back to the lab and placed in plastic shoebox cages (30 × 16 × 9 cm) with Purina Cat Chow®, paper egg cartons (for hiding) and a petri dish (10 cm diameter × 20 cm depth, Nunc) of "lab soil" consisting of a moistened mixture of 50% potting soil and 50% fine sand (by weight). Females were kept in an environmental chamber at 25°C and 14:10 light:dark cycle. Females of both species readily lay eggs into "lab soil" under these conditions (pers. obs.).

Females were allowed to lay eggs for 1 wk. Eggs were retrieved from the petri dishes of soil by washing the soil through a standard sieve set consisting of screens with 1.00 mm, 0.710 mm, and 0.500 mm pores (U.S. Standard Testing Sieve, A.S.T.M. specifications, sizes 18, 25, 35 respectively). Most eggs remained on the 0.710 mm mesh. This procedure recovered >99% of the eggs for each soil type in test runs with known numbers of eggs. Eggs were stored first at room temperature (at least 10 d) then at 4°C in petri dishes lined with moistened filter paper, until needed for the experiments.

Soil Preparation.—For experimental soils, we collected soil from four locations: Sharon 1, Guilford 2, Housatonic Meadows, and Saybrook Point (Fig. 1). These soils were characterized for organic content and particle size distribution, two distinct physical differences occuring between many loams and sands (McKeague 1978). Organic content was determined by weighing dried samples before (dry weight) and after (ash weight) burning in a kiln at 500°C. Particle size distribution was determined using the Hydrometer method (Bouyoucos 1926, Day 1965, Sheldrick & Wang 1993). Based on the USDA (Bureau of Plant Industry, Soils, and Agricultural Engineering) guide for textural classification, soil at Sharon 1 is classified as a loam with a particle size distribution of 44.3% sand, 46.4% silt, and 9.3% clay and an organic content of 7.8%. Soil at
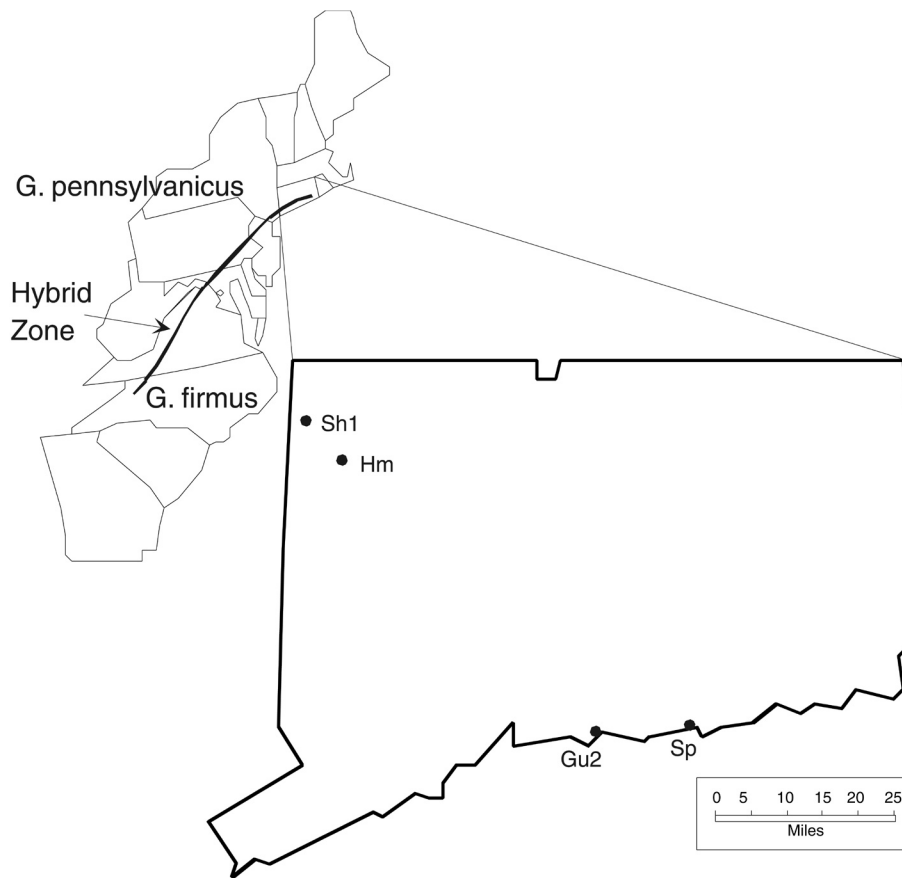
Fig. 1. Location of the field cricket hybrid zone in eastern North America and populations in Connecticut used in this study. Sh1 = Sharon 1, Hm = Housatonic meadows, Gu2 = Guilford 2, Sp = Saybrook Point. All sites correspond to previously collected sites (Harrison 1986, Harrison *et al.* 1997, Harrison & Bogdanowicz 1997, Harrison & Rand 1989). Specific details of locations available from C.L.R.

Guilford 2 is classified as a sand, with a particle size distribution of 95.5%, 3.5%, and 1.1%, for sand silt and clay respectively, and 0.29% organic content. Soil at Housatonic meadows is classified as a sandy loam, with a particle size distribution of 67.7%, 25.75%, and 6.55%, for sand silt and clay respectively, and 6.03% organic content; soil at Saybrook Point is classified as a sand, with a particle size distribution of 86.7%, 10.25%, and 3.05%, for sand silt and clay respectively, and 2.16% organic content. Only the top 10 cm of soil was collected. Females lay eggs only into the top 2 cm of soil (approximately); however, the top 10 cm was collected to ensure an adequate representation of the soil from the parent material in the area, and to average over the inevitably large amount of variation which is typical of the top few centimeters of soil at any site (R. Bryant, pers. com.). Before use, different soil samples from a location were thoroughly mixed and then examined for cricket eggs that may have already been laid in the field.

Experimental Design.—Eggs were placed in nylon mesh containers (25 cm³) with 1-mm pore size. For each container, moistened soil was placed in the bottom of the container and then lightly tamped down with a 20-gm weight to a depth of 3.5 cm. Twenty five eggs total from 3 to 6 females of one population were then placed in the container on top of the soil, at least 1 cm from any side of the container. The eggs were covered with an additional 1.5 cm of moistened soil and then tamped down again with the 20-g weight. Eggs were buried 1.5 cm deep because this is the approximate average ovipositor length across *G. firmus* and *G. pennsylvanicus* populations within the hybrid zone. Egg depth has been shown to affect survival of hatchlings (Bradford et al. 1993, Carrière and Roff 1995). However, separate experiments revealed that eggs buried

at 1.5 cm had the greatest survivorship among those buried at 0.5 cm, 1.0 cm, or 2.0 cm, under the conditions of this experimental design (data not shown).

Containers of eggs were transported to field sites and buried so that the top of the soil within the containers was flush with the surface of the ground. This ensured that the eggs were actually buried 1.5 cm below the ground surface level in the field. The mesh containers allowed for exchange of water, nutrients and organisms with the surrounding environment, while the containers were in the field, but still allowed for easy retrieval of the eggs and soil at the end of the winter.

Three factors were tested in the experiment: the cricket population (nested within species) from which the eggs originated, the soil sample (nested within soil type) used in the container, and the location (nested within region) where the container was buried for the winter (See Ross & Harrison 2006 for details). Two populations of each species (SH1, HM—*G. pennsylvanicus*; GU2, SP—*G. firmus*) were tested in two soil samples of each soil type (SH1, HM—loam; GU2, SP—sand) at two locations within each region (SH1, HM—upland; GU2, SP—coastal). Thirty-two of the possible 64 unique species/soil/location combinations (incomplete block design) were tested with the containers. The combinations that were tested were chosen to maximize comparisons within species, soil types, and regions, while still allowing comparisons among sets of populations, soils, and locations across species, soil types, and regions. Ten replicate treatments were performed for each unique population/soil/location combination, leading to 320 containers. At each location, two spatially distinct subsites were used to control for microhabitat or environmental effects within each location, each consisting of 40 containers.

Table 1. Overwintering egg survival.

bag #	ants?	location	subsite	soil	soil type	cricket population	species	# eggs hatched (of 25)
37	no	SH1	2	HM	loam	SH1	penn	18
38	no	SH1	2	HM	loam	SH1	penn	21
39	no	SH1	2	HM	loam	SH1	penn	19
40	no	SH1	2	HM	loam	SH1	penn	22
251	no	SP	1	HM	loam	HM	penn	20
254	no	SP	1	HM	loam	HM	penn	13
261	no	SP	1	HM	loam	SP	penn	13
263	no	SP	1	HM	loam	SP	firmus	14
265	no	SP	1	HM	loam	SP	firmus	14
281	no	SP	1	GU2	sand	SP	firmus	7
282	no	SP	1	GU2	sand	SP	firmus	11
284	no	SP	1	GU2	sand	SP	firmus	21
285	no	SP	1	GU2	sand	SP	firmus	17
303	no	SP	1	SP	sand	GU2	firmus	22
304	no	SP	1	SP	sand	GU2	firmus	9
305	no	SP	1	SP	sand	GU2	firmus	20
36	yes	SH1	2	HM	loam	SH1	penn	14
252	yes	SP	1	HM	loam	HM	penn	0
253	yes	SP	1	HM	loam	HM	penn	0
255	yes	SP	1	HM	loam	HM	penn	2
262	yes	SP	1	HM	loam	SP	firmus	6
264	yes	SP	1	HM	loam	SP	firmus	6
283	yes	SP	1	GU2	sand	SP	firmus	2
301	yes	SP	1	SP	sand	GU2	firmus	11
302	yes	SP	1	SP	sand	GU2	firmus	14

Containers of eggs were buried at the various sites in Connecticut in early December 1996. Containers buried at this time ensured that no crickets were still alive in the field to contaminate the containers with eggs. The placement of specific egg containers within a grid at each subsite was randomized to avoid any positional bias. Egg containers were allowed to overwinter in the field, then removed from the field in late April 1997, wrapped in Aluminum foil to preserve the structural integrity of each container, and returned to the lab to observe the number of hatchlings that emerged. The presence of ants in containers or ant activity (soil modification) was noted for each container. Containers were placed in an environmental chamber at 25°C with a 14:10 h light:dark cycle and kept moist. The number of hatchlings that emerged from each treatment was recorded. With this design, the experiment measured the combination of overwintering survival of eggs and the ability of first-instar hatchlings to emerge from the soil substrate before desiccation, which is a significant source of mortality in crickets (Bradford *et al.* 1993).

Statistical analysis.—The number of eggs surviving the winter in containers with ants was compared against similar containers (soil, species, location) without ants. For the entire experiment, four main effects were tested: the presence/absence of ants, the species from which the eggs originated (cricket locality), the soil type in the containers (soil locality), and the regional location where the containers were buried (experimental locality). However, because all treatment combinations did not experience ant infestations, which resulted in some empty cells in the experimental design,

a full factorial ANOVA model was not appropriate. Instead, two separate 2-way model III ANOVAs were performed: 1) the presence of ants and species as main effects, including the interaction term, and 2) the presence of ants and soil as main effects, including the interaction term. For appropriate comparisons, in the first ANOVA only containers at the SP location with HM soil were included. For the second ANOVA, containers with HM and SP soil were used, but only with *G. firmus* eggs. The analyses were performed using the PROC MIXED procedure of SAS, with ants as a random effect (SAS Institute, Inc. 1997) and the statistical packages, JMP 5.01 (SAS Institute, Inc. 2002) and Data Desk 6.2 (Data Description Inc. 2003). From an initial analysis of the data, every location showed little variation between subsites. As a result, subsites were grouped within any location for subsequent analyses.

Results

Cricket eggs hatched nearly synchronously within each treatment 2 to 3 wk after removal from the field. Over all containers, 54% of eggs hatched. From a total of 320 egg containers, nine containers showed activity or the presence of ants. These containers and containers without ants, but with equivalent treatment effects (soil type, location, species), were used for subsequent analysis for a total of 25 containers (Table 1). Both two-way ANOVAs reveal that ants induce significant mortality for cricket eggs (Table 2): fewer eggs emerged from containers with ant disturbance (6.1 eggs [26%] ± 1.9) than when no ants were present (16.3 eggs [66%] ± 1.2). Neither two-way interaction term, of ants by soil type or species,

Table 2. Egg survival ANOVAs.

A. Ants × Species

Source	DF	Type III Sum of Squares	F ratio	Prob. > F
Ants?	1	308.267	52.347	0.0004
Species	1	9.600	0.360	0.6560
Ants? × Species	1	26.667	4.538	0.0774
Error	6	35.333		
Total	9	431.600		

B. Ants × Soil type

Source	DF	Type III Sum of Squares	F ratio	Prob. > F
Ants?	1	258.403	11.856	0.0033
Soil type	1	53.669	0.945	0.5090
Ants? × Soil type	1	56.803	2.606	0.1260
Error	16	348.722		
Total	19	965.800		

was significant, although the interaction of ants × species shows a strong trend (Table 2).

Results from the companion experiment testing the effects of soil, location, species and winter weather, indicated that all of these main effects significantly affect the survival and hatching of overwintering cricket eggs (Ross & Harrison 2006). In this experiment, more eggs survived in upland areas than coastal areas (14.6 eggs [58.4%] ± 0.48 *vs* 9.6 eggs [38.4%] ± 0.49), more *G. pennsylvanicus* eggs survived compared with *G. firmus* eggs (13.1 eggs [52.4%] ± 0.49 *vs* 11.1 eggs [44.4%] ± .48), more eggs survived when they overwintered in sand than loam (12.9 eggs [51.6%] ± 0.48 *vs* 11.3 eggs [45.2%] ± 0.48), and more eggs survived in the winter of 1996-97 than 1993-94 (14.8 eggs [59.2%] ± 0.36 *vs* 9.4 eggs [37.6%]± 0.58) (Ross & Harrison 2006). However, two-way interactions also were significant, and these interactions reveal why each of the main effects were significant and produce three main conclusions. First, the significant main soil effect is the result of more variation in the survivorship of eggs in sand across regions and years, than in loam, and not a result of differences across species. Second, egg survival differed greatly across the two winters for the two cricket species. Third, more eggs survived at upland sites than coastal sites under all soil, weather, and species treatments (Ross & Harrison 2006).

The subset of data involving ant activity that is presented here is consistent with those results in one case, but not in another case (Table 2, Fig. 2). Egg survival at the upland site was greater than at the coastal site (upland: 18.8 eggs ± 3.11; coastal: 11.1 eggs ± 1.56; t = 2.38, p = 0.026). Egg survival in sand was not more variable than in loam (std dev: sand = 6.59, loam = 7.58; Levene F-ratio = 0.2899, n.s.). However, unbalanced sample sizes for different treatments in this comparison make this comparison weak. Ant infestations only occurred in one winter of the experiment, so variation in egg survival could not be evaluated across winters.

Inspection of the containers with ant activity indicated extensive tunneling through the containers and other soil modifications, such as physical properties of the soils. Fine inspection of soils in the containers revealed no unhatched cricket eggs or broken egg casings. However, no unhatched eggs or egg casings were found in containers where no ant activity was observed, so the lack of these materials probably is not a result of ant infestations.

Discussion

Hybrid zone pattern and process.—In order to understand the dynamics of a hybrid zone, we must investigate both the patterns that structure the zone as well as the processes that lead to its maintenance. These factors are important to the evolution that occurs in a hybrid zone, because structure and maintenance provide the opportunity for individuals and alleles to interact, and the criteria on which those interactions are judged by natural selection. Two different hybrid zone structures for example, strongly influence the ability of two incipient species to develop reproductive isolation through reinforcement. In mosaic hybrid zones, the likelihood of prezygotic barriers to genetic exchange developing as a result of reinforcement is greater than in simple, clinal hybrid zones (Cain *et al.* 1999). Mosaic zones provide more opportunity for species to interact, while the mosaic nature of the hybrid zone allows these many interactions to be "semi-independent" of each other, due to restricted (but not zero) gene flow among like patches (Cain *et al.* 1999). As a result, reinforcement is more likely to develop over a wider set of conditions. Mosaic hybrid zones also increase the likelihood of persistence and divergence of parental types, even at low levels of hybrid disadvantage (Sadedin & Littlejohn 2003). Clinal hybrid zones usually cannot provide this complexity of interaction and therefore are less likely to lead to persistence, reinforcement, and divergence. Additionally, mosaic hybrid zones can sustain genotypes that are disfavored in numerous habitats, when the structure of the zone allows for these genotypes to frequently "escape" to new uninhabited patches, given temporal heterogeneity in patch creation and extinction as with a metapopulation model (Hanski 1983, Durrett *et al.* 2000, Keeling *et al.* 2003).

The processes involved in the maintenance of mosaic hybrid zones may result from intrinsic (hybrid dysfunction) and extrinsic (environmental) mechanisms. Though intrinsic mechanisms likely are important in both clinal and mosaic hybrid zones, extrinsic factors play a major role in maintaining mosaic hybrid zones (Barton & Gale 1993, Harrison 1993, Arnold 1997). Habitat segregation to alternate habitat patches in these hybrid zones, not only structures the zones, but is also the mechanism for their maintenance.
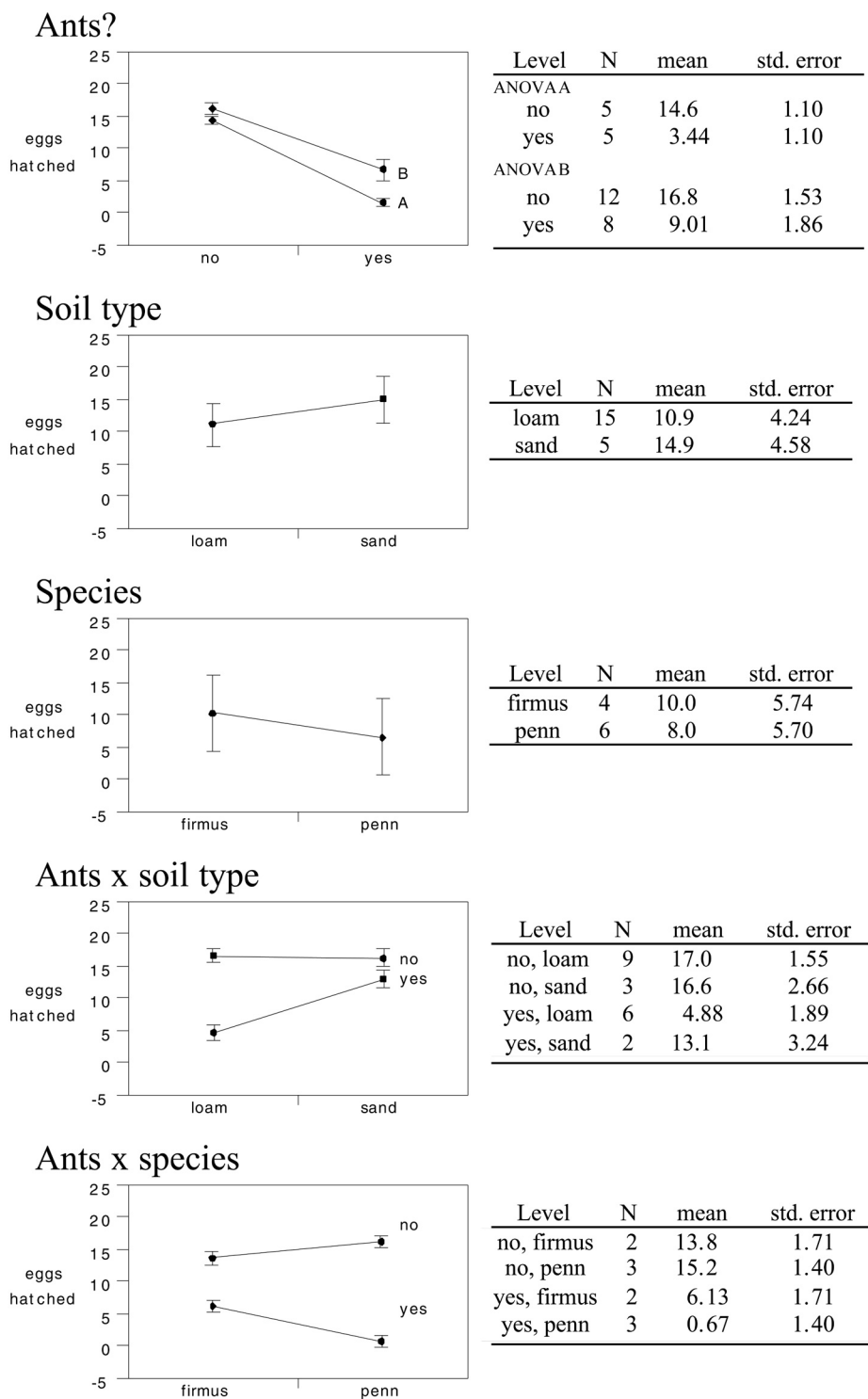
Ants?

Level	N	mean	std. error
ANOVA A			
no	5	14.6	1.10
yes	5	3.44	1.10
ANOVA B			
no	12	16.8	1.53
yes	8	9.01	1.86

Soil type

Level	N	mean	std. error
loam	15	10.9	4.24
sand	5	14.9	4.58

Species

Level	N	mean	std. error
firmus	4	10.0	5.74
penn	6	8.0	5.70

Ants x soil type

Level	N	mean	std. error
no, loam	9	17.0	1.55
no, sand	3	16.6	2.66
yes, loam	6	4.88	1.89
yes, sand	2	13.1	3.24

Ants x species

Level	N	mean	std. error
no, firmus	2	13.8	1.71
no, penn	3	15.2	1.40
yes, firmus	2	6.13	1.71
yes, penn	3	0.67	1.40

Fig. 2. One- and two-way interaction plots for comparisons in ANOVAs (Table 2). For each plot corresponding tables list: level, sample size for ANOVA cell, and least square mean estimates (± standard error). For the "Ants?" plot, estimates from both ANOVAs are shown.

This partitioning of habitats may result from antagonistic selection between these patches (Endler 1981, Nürnberger *et al.* 1995), behavioral preferences for one habitat over the other (MacCallum *et al.* 1998, Pearson 2000), or potentially—competition between parental types (Pearson & Rohwer 2000). Elucidating these forces will provide insight into the maintenance of the zone, as well as the criteria for differential introgression of specific traits (and those genetically linked to them). That is, trait differences that influence local distribution within mosaic hybrid zones contribute to species coexistence and persistence in the face of hybridization, and thus habitat segregation can serve as a barrier to gene exchange as well as playing a prominent role in structuring and maintaining the hybrid zone.

Pattern and process in the Gryllus hybrid zone.—In the *Gryllus* hybrid zone, multiple mechanisms are involved in the structure and maintenance of the zone. Although no distinct intrinsic mechanisms for hybrid zone maintenance have been identified for this system, a number of pieces of evidence suggest the existence of some barriers to hybridization. Both species may employ prezygotic barriers in the form of partial assortative mating or selective fertilization (Harrison 1983, R. Harrison, pers. comm.). Extrinsic factors as well may contribute to partial reproductive and genetic isolation between species in the *Gryllus* mosaic hybrid zone. As soil type plays a dominant role in structuring this hybrid zone, it likely is involved in its maintenance as well. These crickets have an intimate relationship with the soil because females use it as an oviposition substrate and, in Connecticut, diapausing eggs must overwinter in the soils. Consequently, understanding how these species differ in their interaction with different soil types—specifically as eggs—should shed light on barriers to hybridization in the hybrid zone. Of three possible factors driving soil type segregation in this hybrid zone — behavior, selection, and competition — interspecific competition driving habitat segregation in these crickets is unlikely. Interspecific competition is rare in detritivores where resources generally are not limiting (Alexander 1968; Harrison 1978; Schoener 1983; though see Giller & Doube 1989, 1994).

Different oviposition preferences for different soil types by each species may lead to geographic isolation by soil types and strengthen barriers between these crickets. Indeed, *G. pennsylvanicus* does have a strong preference to lay eggs in loam soils, the habitat where it is found. However, *G. firmus* also has a strong preference to lay eggs in loam, even though it is found on sandy soils within and outside of the hybrid zone (unpub. data). *G. pennsylvanicus'* preference is strong and inflexible (females will not lay eggs in sand), but *G. firmus'* preference is less rigid (it will lay eggs in sand or loam). Therefore, this behavior cannot fully explain the geographic isolation and habitat segregation we see in the hybrid zone or between these species, and thus it cannot be a complete barrier to hybridization.

Similar to a behavioral preference for soil type, differential selection by different soil types may explain the habitat segregation we see between these crickets and thus act as an extrinsic barrier to hybridization. The companion study to this one (Ross & Harrison 2006) tested viability selection on overwintering eggs in a number of natural conditions in the *Gryllus* hybrid zone. While soil type was an important selective agent for cricket egg survival, *G. pennsylvanicus* and *G. firmus* did not differ in their survival based on soil type. Regional characteristics (upland *vs* coastal areas) and variation in winter weather had stronger effects on egg viability than soil type, indicating that nonsoil habitat factors may play an important role in structuring and maintaining this hybrid zone.

The results presented here demonstrate that one nonsoil ecological factor, the presence of ants, constitutes a strong selection pressure on the survival of overwintering eggs. Egg viability was reduced 2.5-fold when ants interacted with cricket eggs. Although no other effects in the ANOVA models were significant, the occurrence of ants in specific environmental conditions suggest this selection pressure is not uniform throughout the hybrid zone spatially or temporally. From Table 1, ant infestations occurred only in two (SP, SH1) of the four sites where the experiment was performed, and most ant interactions occurred at only one site (SP). In absolute numbers, the effect of ants on egg viability was more severe at SP than SH1, to the extent that egg survival was near zero for most containers at SP. A notable exception at SP was the relatively low mortality of *G. firmus* eggs buried in SP soil (sand) at this site. It is unclear why eggs in this experimental condition did not experience more mortality. Moreover, this experiment was performed over two different winters (1993-94, 1996-97), yet ant infestations were present only in the 1996-97 winter. Climate data from the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center database (NOAA online database 2005) indicate that in Connecticut the 1996-97 winter was the 7th warmest on record (1895-2005), while the 1993-94 winter was typically cold for this region (98th warmest). The presence of ant activity in 1996-97 suggests that ant predation is facilitated by relatively warm winters when these ants become active earlier in the season before cricket eggs normally break diapause. This also would explain why more ant activity was seen at SP, the warmer coastal site, than SH1, the colder upland site.

Variation in predators among sites, soils, and years may be specific to the species of concern, in this case the ant, *Lasius neoniger*. This species is very common in eastern North America, but prefers open, frequently disturbed and sandy areas, such as SP (Wilson 1955, Wang *et al.* 1995). These mound-building ants typically make shallow, interconnected nests in the first 30 cm of soil, overlapping with the same soil layers "(top 2 cm)" as diapausing cricket eggs (Wang *et al.* 1995). It is not clear whether these ants discover and predate eggs through chance encounters in the soil or through active predation, though their extensive soil displacement and modification suggest that an ant colony near cricket eggs in the soil would have a high probability of encountering these eggs. It is not apparent why egg mortality was not 100% in this case. These ants also may alter the spatial distribution of soil nutrients through physical soil modification (Wagner *et al.* 2004). Physical modification of soils near diapausing cricket eggs may lead directly to mortality due to changing exposure to abiotic factors, such as winter weather. The depth eggs are laid in soils has been shown to be critical for overwintering egg survival in crickets (Lutz 1908; Masaki 1979, 1986; Bradford *et al.* 1993; Mousseau and Roff 1995; Carrière *et al.* 1997).

Survival of eggs in soils at various depths is affected by competing factors. Eggs at relatively shallow depths experience more mortality to freezing and desiccation due to lack of soil as an insulator, whereas hatchlings from eggs buried too deep cannot reach the surface before death (Masaki 1979, 1986; Bradford *et al.* 1993; Mousseau and Roff 1995; Carrière *et al.* 1997). Consequently, physical soil modification may have led to more variation and greater exposure to abiotic environmental factors that affect egg survival. This, instead of ant predation, may explain why egg mortality was not 100% for containers infested with ants.

Acknowledgements

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