

Multiple mechanisms contribute to isolation by environment in the redheaded pine sawfly, *Neodiprion lecontei*

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Abstract

Isolation by environment (IBE) is a population genomic pattern that arises when ecological barriers reduce gene flow between populations. Although current evidence suggests IBE is common in nature, few studies have evaluated the underlying mechanisms that generate IBE patterns. In this study, we evaluate five proposed mechanisms of IBE (natural selection against immigrants, sexual selection against immigrants, selection against hybrids, biased dispersal, and environment-based phenological differences) that may give rise to host-associated differentiation within a sympatric population of the redheaded pine sawfly, *Neodiprion lecontei*, a species for which IBE has previously been detected. We first characterize the three pine species used by *N. lecontei* at the site, finding morphological and chemical differences among the hosts that could generate divergent selection on sawfly host-use traits. Next, using morphometrics and ddRAD sequencing, we detect modest phenotypic and genetic differentiation among sawflies originating from different pines that is consistent with recent, in situ divergence. Finally, via a series of laboratory assays—including assessments of larval performance on different hosts, adult mate and host preferences, hybrid fitness, and adult eclosion timing—we find evidence that multiple mechanisms contribute to IBE in *N. lecontei*. Overall, our results suggest IBE can emerge quickly, possibly due to multiple mechanisms acting in concert to reduce migration between different environments.

Keywords: isolation by environment, host-associated differentiation, genetic differentiation, ecological speciation, divergence with gene flow, reproductive barriers

Introduction

Isolation by distance (IBD)—a pattern in which genetic similarity declines as a function of geographic distance between individuals—is one of the most ubiquitous population genetic patterns in nature (Meirmans, 2012; M. A. Peterson & Denno, 1998; Sexton et al., 2014). The primary mechanism generating this pattern is geographically restricted dispersal: As gene flow declines between increasingly distant locations, genetic differentiation can accumulate via drift (Wright, 1943, 1946). Independent of geographical distance, environmental differences can also restrict gene flow between locally adapted populations via multiple mechanisms, such as biased dispersal and natural and sexual selection against immigrants and hybrids. When this occurs, individuals from dissimilar environments will tend to be more genetically differentiated than individuals from similar environments, a pattern called isolation by environment (IBE) (Bradburd et al., 2013; Sexton et al., 2014; Wang & Bradburd, 2014; Wang & Summers, 2010).

Compared to a rich literature documenting both IBD patterns (Battaglia et al., 2008; Jauker et al., 2009; Moore et al., 2008; Svenning et al., 2008; Sydenham et al., 2017) and mechanisms that cause geographically restricted dispersal (Baguette & Van Dyck, 2007; Bowler & Benton, 2005; Clobert et al., 2009; Matthysen, 2013; Pflüger & Balkenhol, 2014; Ronce & Clobert, 2013), research into IBE is more limited. Nevertheless, a growing number of studies are finding evidence of IBE (e.g., Bagley et al., 2017; Mancilla-Morales et al., 2022; Moncada et al., 2021; Prunier et al., 2017), raising the possibility that this pattern is just as ubiquitous—if not more so—than IBD (Sexton et al., 2014; Shafer & Wolf, 2013). Still, major challenges persist when studying IBE. First, geography and ecology are often strongly correlated, making it difficult to disentangle their individual effects. Several approaches have been proposed to account for this spatial autocorrelation (e.g., Mantel and partial Mantel tests [Mantel, 1967; Smouse et al., 1986; Sokal, 1979], BEDASSLE [Bradburd et al., 2013], MMRR [Wang, 2013], SUNDER

Received May 22, 2023; revisions received July 17, 2023; accepted July 21, 2023

Associate Editor: Marcus Kronforst; Handling Editor: Miriam Zelditch

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[Botta et al., 2015], distance-based redundancy analysis [Driscoll et al., 2019]), but their application remains limited. Second, and perhaps more importantly, detection of an IBE pattern does not in itself reveal which of several non-mutually exclusive mechanisms gave rise to the observed pattern. However, few studies have gone beyond documenting IBE to test underlying mechanisms.

Wang and Bradburd (2014) described four potential mechanisms that may generate the IBE pattern via reducing effective migration between divergent environments. First, when populations are locally adapted to a specific environment, individuals from that population may fare poorly when they disperse to an alternative environment (Kawecki & Ebert, 2004; Nosil et al., 2005; Zhang et al., 2017; also see Kawecki, 1997). This selection against nonlocally adapted immigrants will tend to decrease gene flow between dissimilar habitats (Crispo et al., 2006; Wang et al., 2013). Second, immigrants may be sexually selected against if they originated from a source population experiencing divergent sexual selection. Sexual selection against immigrants can act alongside natural selection, for example if the choosy sex tends to prefer individuals with traits well suited to the local habitat (Ingleby et al., 2010; Jia & Greenfield, 1997; Nosil et al., 2005). Immigrants from other host plants may also be unattractive mates if their diet influences chemical mating signals, such as their cuticular hydrocarbon profile (e.g., Etges & Tripodi, 2008; Kühbandner et al., 2012; Rundle et al., 2005). Alternatively, sexual signals themselves may be optimized for local environments, thereby reducing signaling efficacy—and reproductive success—when individuals display these signals in different environments (e.g., Boughman, 2001; Pires et al., 2019; Seehausen et al., 2008). A third mechanism that can produce an IBE pattern is when natural or sexual selection acts against hybrid offspring produced by parents from different environments. Hybrid individuals may, for example, exhibit an intermediate phenotype unsuitable for either parental habitat, reducing survival or opportunities for attracting mates (e.g., Chhina et al., 2022; Jacquemyn et al., 2018; McBride & Singer, 2010). Finally, IBE can be generated if individuals are more likely to disperse to a similar environment than to a different environment, either via genetically based habitat preferences (e.g., Bolnick & Otto, 2013; Edelaar et al., 2008; Feder & Forbes, 2007) or via plastic responses to the natal habitat (Benard & McCauley, 2008; J. M. Davis & Stamps, 2004; Merrick & Koprowski, 2016).

In addition to the four IBE mechanisms described by Wang and Bradburd (2014), habitat-related differences in phenology can also produce an IBE pattern (Sexton et al., 2014). Such differences can arise via two non-mutually exclusive routes. First, phenological differences among populations in different environments could result from divergent selection and local adaptation. For example, in the apple maggot fly, *Rhagoletis pomonella*, heritable differences in adult eclosion time synchronize host races with the availability of ripe fruit for oviposition, which differs among host plants (Doellman et al., 2018; Feder et al., 1993, 1994). Second, even in the absence of genetic differences in developmental timing, developmental plasticity in response to environmental variables could give rise to differences in reproductive periods between populations living in different habitats. For example, shifts in flowering time may arise in plants occupying niches with differing environmental conditions (e.g., temperature, pH, moisture level) that affect plant physiology (Gavrilets &

Vose, 2007; Levin, 2009; Rafferty et al., 2020; Silvertown et al., 2005). Regardless of the source of the phenological shift, when populations differ in the timing of their reproductive periods, gene flow will be reduced between dissimilar environments and a pattern of IBE can be produced (Boumans et al., 2017; Taylor & Friesen, 2017; Y. M. Zhang et al., 2018).

Because all IBE mechanisms ultimately reduce gene flow between populations, understanding how and when they contribute to divergence may provide insight into the conditions that facilitate divergence with gene flow (Butlin & Smadja, 2018; Smadja & Butlin, 2011). Moreover, documenting IBE patterns and mechanisms has clear relevance to understanding local adaptation and speciation. However, detection of the IBE pattern alone is not sufficient to determine that local adaptation or incipient ecological speciation is occurring because some IBE mechanisms do not involve divergent selection or heritable trait variation (Wang & Bradburd, 2014). Understanding the relationship between IBE and ecological speciation, therefore, requires assessing the relative importance of IBE mechanisms across diverse taxa and divergence scenarios. But in contrast to the rich body of ecological speciation literature documenting how divergent selection produces reproductive isolation (Nosil, 2012)—particularly in plant-feeding insects (Forbes et al., 2017; Matsubayashi et al., 2010)—very few studies have evaluated IBE mechanisms at all, let alone multiple potential IBE mechanisms in the same system (Wang & Bradburd, 2014).

In this study, we evaluate multiple IBE mechanisms in *Neodiprion lecontei*, an experimentally tractable species for which an IBE pattern was previously detected (Bagley et al., 2017). *Neodiprion* (Hymenoptera: Diprionidae) is a Holarctic genus of sawflies that specialize on pines. Like many plant-feeding insects, *Neodiprion* sawflies are closely associated with their host plants throughout their life cycle: Adults mate on the host plant, females lay eggs into pockets cut within host needles, and larvae consume the needles during development before spinning cocoons on or beneath the host (Benjamin, 1955; Coppel & Benjamin, 1965; Knerer, 1993; Wilson et al., 1992). Most species also feed on only one or a small handful of host plant species (Linnen & Farrell, 2010; Smith, 1993). Due to this high degree of specialization and intimate, lifelong relationship with their host plants, it has long been hypothesized that host adaptation is a primary driver of population differentiation and speciation in *Neodiprion* sawflies (Alexander & Bigelow, 1960; Bush, 1975a, 1975b; Ghent & Wallace, 1958; Knerer & Atwood, 1972, 1973). Consistent with this hypothesis, changes in host use are associated with speciation events in the genus (Linnen & Farrell, 2010), and divergence in host-use traits contributes to both prezygotic isolation (Glover et al., 2023) and extrinsic postzygotic isolation (Bendall et al., 2017).

Although *N. lecontei* is a pine generalist compared to other species in the genus (Linnen & Farrell, 2010; Wilson et al., 1992), *N. lecontei* populations collected from different pine species tend to be more genetically dissimilar than those collected from the same pine species, after controlling for historical isolation and geographic distance (Bagley et al., 2017). To understand why this pattern exists, we use field observations and laboratory experiments to evaluate potential IBE mechanisms in *N. lecontei*. To remove the effect of geography entirely, we focus on a single location where *N. lecontei* was observed feeding on three different *Pinus* species. To evaluate potential host-related sources of divergent selection,

we first characterize the morphology, volatile chemistry, and resin content of the three *Pinus* hosts. To characterize population structure and evaluate IBE at this single site, we generate genome-wide genetic data via double-digest restriction-associated DNA (ddRAD) sequencing. Finally, to understand mechanisms that give rise to host-associated differentiation (a specific type of IBE) among *N. lecontei* collected on three pine species, we evaluate (a) host-based performance differences (natural selection against immigrants), (b) mate preferences (sexual selection against immigrants), (c) hybrid survival (natural selection against hybrids), (d) female host preferences (habitat-based dispersal bias), and (e) adult eclosion patterns (habitat-related differences in phenology). Taken together, our results suggest that multiple IBE mechanisms can and do act in concert, possibly facilitating its rapid emergence in natural populations.

Materials and methods

Study site and host trees

Our study site was located at the University of Kentucky's Arboretum and State Botanical Gardens, established in 1991 in Lexington, KY. Spanning a transect of ~130 m, the "Trail of Pines" (38.0167°N, 84.5047°W) has three pine species: *Pinus echinata* (shortleaf pine), *P. virginiana* (Virginia pine), and *P. rigida* (pitch pine). The trees were planted in the mid-to-late 1990s (T. Rounsaville, personal communication) near each other, with the branches of some trees physically intermingling (Supplementary Figure 1). The three pine species are all native to Kentucky and can be found in national forests and other nature preserves within the state (e.g., in Daniel Boone National Forest, which is ~100 km from the site) but are not common in the Lexington area. The primary study period was between 2012 and 2015, during which *N. lecontei* larvae were abundant on all three species. Although we do not know exactly when sawflies first colonized the study site, given the age of the trees, the Trail of Pines population of *N. lecontei* was likely no more than ~10–15 years old (and possibly much younger) at the start of the study. With two to three generations per year, on average, in Lexington (R.K.B. and C.R.L., personal observation), the maximum age of the population is 30–45 generations.

To gain insight into potential host-related selection pressures that could give rise to IBE among sawflies feeding on the three different pine species, we described morphological and chemical differences between the pine species. First, because needle width affects oviposition success of *N. lecontei* females (Bendall et al., 2017; Glover et al., 2023), we asked whether needle width differed among the three pine species. From each individual pine tree in the Trail of Pines (*P. echinata* $N = 4$, *P. rigida* $N = 2$, *P. virginiana* $N = 3$), we measured the width of 10 needles with digital calipers (Mitutoyo CD-6"PMX). Because we used greenhouse-grown seedlings of these same species in our assays (see below), we also measured the width of seedling needles so that we could make comparisons between trees at our collection site (potential sources of divergent selection on wild-caught sawflies) and the seedlings used in our assays. For each of the three species of pine, we measured 10 needles from each of 10 randomly selected seedlings.

We analyzed the needle data—and, unless otherwise noted, all other data—in R version 4.2.1 (R Core Team, 2022). We used the *lmer* function (lmerTest v 3.1-3; Kuznetsova et al., 2017) to fit a mixed-effect model to the needle width

measures, with individual tree as a random effect and pine species, life stage, and their interaction as fixed effects. We used a Type III analysis of variance (ANOVA) to assess the significance of the fixed effects, followed by the *emmeans* function (emmeans v1.8.0; Lenth, 2020) for post hoc pairwise comparisons among (a) pairs of pine species, (b) life stages within pine species, and (c) pairs of pine species within life stages. All post hoc comparisons used Benjamini–Hochberg correction for multiple testing. This and all other R code can be found on DRYAD (Bagley et al., 2023).

Next, to determine whether volatile profiles differed among hosts, which could facilitate divergence in female host preferences, we collected the volatiles emitted from trees planted along the Trail of Pines ($N = 4$ samples collected for each host). We conducted headspace sampling by enclosing a set of needles in a caprolactum bag and loosely securing the open end. Volatile collection, extraction, and identification via GC–MS followed the approaches outlined in Frost et al. (2007, 2012) and Frost (2023), and more details can be found in Supplementary Methods. We compared the total volatile profiles using non-metric multidimensional scaling (NMDS), a robust ordination method that has been commonly applied to the multivariate analysis of volatile profiles (Bricchi et al., 2010; Minchin, 1987). NMDS was performed using the *metaMDS* function (vegan v2.6-4; Oksanen, 2010), with 95% confidence ranges generated using *veganCovEllipse*. For analysis of individual volatiles, we fit a linear model for each compound with tree species as the fixed factor. We used a Type II ANOVA (car v3.1-1; Fox & Weisberg, 2019) to assess the main effect across the three pine species, followed by post hoc pairwise comparisons.

Finally, to determine whether resin content differed among the three pine species, which could differentially impact larval growth and survival (Larsson et al., 1986), we quantified resin content from branch clippings collected from exemplars of each species. These clippings were collected as a part of a broader survey of pine traits in eastern North America (Glover et al., 2023), but we focus specifically here on samples taken from *P. echinata*, *P. virginiana*, and *P. rigida*. For each tree species, we sampled 10 clippings each from three to four geographically widespread locations (Supplementary Table S1). We sampled each site in May (8 May 2017–21 May 2017) and August (3 August 2017–15 August 2017), both months during which *N. lecontei* can be found in the field (Wilson et al., 1992). After collection, clippings were placed into individual plastic bags and stored on ice until taken to the lab. Upon return to the lab, clippings were stored at 4 °C until resin data were collected.

We quantified total nonvolatile resin content using methods adapted from Moreira et al. (2012, 2014). Additional details on our resin extraction protocol can be found in Supplementary Methods. To determine whether resin content differed among the three host species, we fit a linear model to the resin content data, with host species, month, and their interaction as predictors. Because model residuals were not normally distributed for untransformed data, we fit a linear model to square-root transformed resin data. To evaluate the significance of model terms, we used Type III ANOVA. We then performed post hoc comparisons that evaluated whether there were differences in resin content among pairs of host species either (a) without accounting for sampling period or (b) within each of the two sampling periods, with the Benjamini–Hochberg method for adjusting *p*-values.

Sawfly sampling and propagation

Sawfly colonies (i.e., distinct clusters of feeding larvae) were collected from *P. echinata*, *P. rigida*, and *P. virginiana* at the field site between 2012 and 2015 as early-to-late instar feeding larvae (Supplementary Table S2). A subset of larvae from some colonies was preserved in 100% ethanol for population genetic analysis. The remaining larvae were returned to the lab and reared in plastic boxes (32.4 cm × 17.8 cm × 15.2 cm), with mesh lids and provided clippings of their source host species *ab libitum*. Cocoons were collected three times weekly and stored in individual gelatin capsules until emergence. Larvae and cocoons were kept in walk-in environmental chambers maintained at 22 °C and an 18:6 light-dark cycle. Cocoons were checked daily for emergence, and live adults (which are nonfeeding) were stored at 4 °C to prolong life until needed for propagation or experimental assays.

For IBE mechanism assays (see below; Figure 1), we established lab lines from larval colonies that were originally collected from each host species and reared them for an additional one to two generations on a common non-natal host in the lab. Hereafter, sawfly lines will be referred to by the common name of source host, that is, sawflies collected from *P. echinata* as “Shortleaf,” from *P. rigida* as “Pitch,” and from *P. virginiana* as “Virginia”; the scientific names will exclusively refer to host plants. Briefly, each sawfly line

was produced by releasing male and female adults reared from multiple larval colonies (to maximize genetic diversity) into mesh cages containing multiple seedlings of a host that does not occur in Kentucky, *P. banksiana* (jack pine). The adults were allowed to mate and oviposit freely. Upon hatching, larvae from these cages were transferred into plastic boxes and reared as described above on clippings of field-collected *P. banksiana*. We reared all colonies on the same host species to control for the impact of rearing host on host-related phenotypes. We chose *P. banksiana* as the shared host because it is a primary host for *N. lecontei* (Wilson et al., 1992), is a suitable host for most *Neodiprion* species (Knerer, 1984), and because seedlings of this host could be purchased year-round.

Assessment of population structure and genomic differentiation

DNA extraction, library preparation, and genotyping

A large data set of SNP markers was prepared using the same extraction and double-digest RAD (ddRAD) sequencing approaches described in Lindstedt et al. (2022) and Bendall et al. (2022). Briefly, libraries from 58 Arboretum individuals were prepared following a modified version of the original ddRAD protocol (B. K. Peterson et al., 2012) and labeled with one of 48 unique, variable-length (Burford

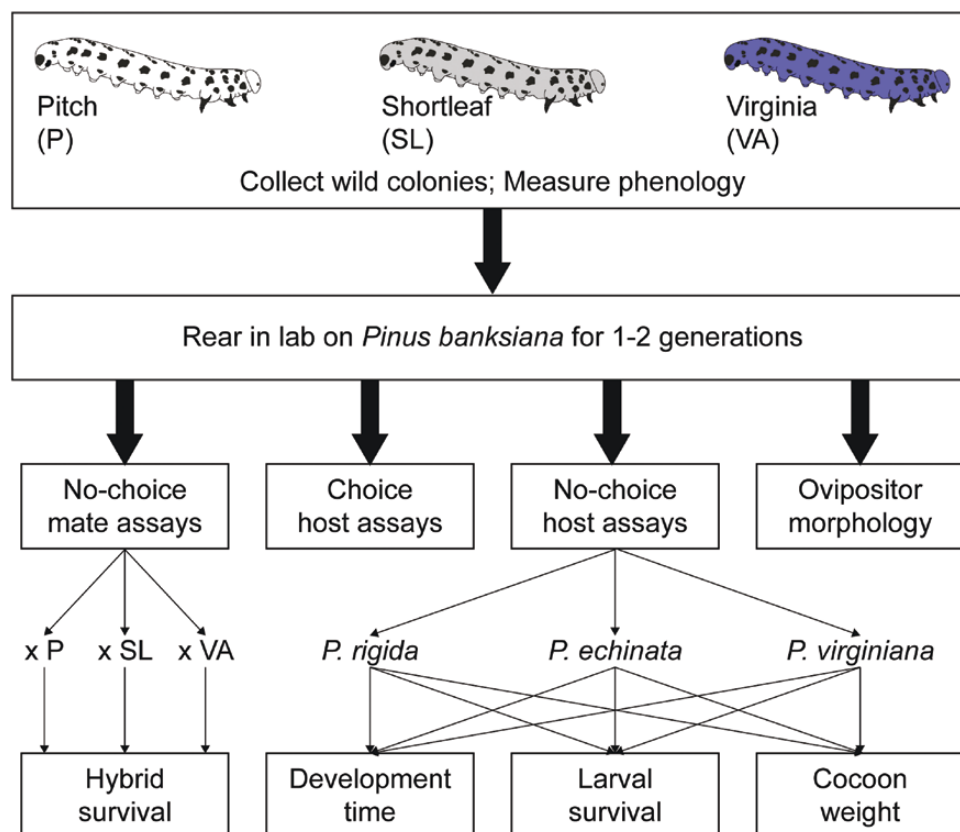


Figure 1. Overview of isolation-by-environment (IBE) mechanism assays. All individuals used in our assays were collected at the “Trail of Pines” field site on *Pinus rigida* (common name: pitch pine; sawflies collected from *P. rigida* = “Pitch” line = P, shown in white throughout manuscript), *P. echinata* (common name: shortleaf pine; sawflies collected from *P. echinata* = “Shortleaf” line = SL, shown in gray throughout manuscript), or *P. virginiana* (common name: Virginia pine; sawflies collected from *P. virginiana* = “Virginia” line = VA shown in blue throughout manuscript), and then reared as described in the text in the laboratory for one to two generations on a common, nonlocal host (*P. banksiana*, common name: jack pine) before being used in various assays. Arrows are drawn to indicate the flow of individuals and their offspring into and between assays.

Reiskind et al., 2016) in-line barcodes during adapter ligation (Supplementary Tables S3 and S4). Barcoded libraries were pooled, size selected (average fragment size = 379 ± 76 bp), and amplified using multiplex read indices (Supplementary Tables S3 and S5). We also included a string of four degenerate bases next to the Illumina read index to allow for the detection of PCR duplicates (Schweyen et al., 2014). Two lanes of 150-bp single-end reads from an Illumina HiSeq 4000 were obtained for the libraries at the High-Throughput Sequencing and Genotyping Unit at the University of Illinois.

Raw sequence reads were quality filtered and trimmed using the *process_radtags* module in STACKS (v1.46; Catchen et al., 2013). Surviving reads were then aligned to a high-coverage, scaffolded genome assembly for *N. lecontei* (Vertacnik et al., 2016; Linnen et al., 2018; coverage: 112x; scaffold N50: 244 kb; GenBank assembly accession: GCA_001263575.1) using BOWTIE2 (v2.3.1; Langmead and Salzberg 2012). Uniquely mapping, high-quality reads (MAPQ ≥ 30) were extracted with SAMTOOLS (v1.3; Li et al., 2009), and putative PCR duplicates were removed. RAD loci were then constructed using the *ref_map.pl* STACKS pipeline (v1.46; Catchen et al., 2013).

After an initial round of SNP calling, we evaluated ploidy and missing data levels using VCFtools (v0.1.14b; Danecek et al., 2011) and excluded seven individuals missing data at $>60\%$ of SNP loci and two putative haploid individuals (Supplementary Table S3). Our final RAD data set consisted of 49 individuals (15 Shortleaf, 13 Pitch, and 21 Virginia). We applied several additional filters to these individuals, excluding all sites missing data in 30% or more of individuals and all sites with a minor allele frequency less than 0.05. We also excluded sites violating Hardy–Weinberg equilibrium for heterozygote excess significant at the 0.01 level, as these sites likely represent sequencing error (Chen et al., 2017). Finally, to minimize linkage disequilibrium between SNPs, we included only one randomly selected SNP per RAD locus. Data processing and all other bioinformatic analyses were performed on the University of Kentucky's Lipscomb High-Performance Computing Cluster.

Discrete population structure analyses

To evaluate the possibility that our study population was seeded by host-specialized populations that diverged elsewhere and were already genetically distinct, we asked whether there was any evidence of discrete population structure. We used the maximum-likelihood-based clustering algorithm implemented in the program ADMIXTURE (v1.3.0; Alexander et al., 2009) to determine the proportion of ancestry for each individual from K ancestral populations without a priori designation. We performed 100 independent runs for values of K from 1 through 10. The optimal K was selected as described in the ADMIXTURE manual, by comparing the fivefold cross-validation (CV) error across different values of K . To determine assignment stability and visualize primary and secondary solutions across the 100 replicates of each K , we used the main pipeline of CLUMPAK (v1.1; Kopelman et al., 2015).

IBE: Host-associated genetic differentiation

Having found no evidence of discrete population structure indicative of previous isolation among the different host lines (see Results), we next asked whether there was any evidence of host-associated differentiation, a specific type of IBE. To

do so, we used custom R scripts (available on DRYAD) to compute the Hudson estimator of F_{ST} (Hudson et al., 1992) for each pair of sawfly lines, following Bhatia et al., 2013. To evaluate the significance of observed F_{ST} estimates, we permuted individuals among populations, recalculating F_{ST} in each permutation. p -Values were calculated from the proportion of 10,000 permutations that had F_{ST} values greater than or equal to the observed F_{ST} values.

Host-associated morphological differences

To complement the genetic data, we asked whether there was evidence of morphological divergence among the three host lines. We focused specifically on ovipositor size and shape because ovipositor morphology has been linked to egg-laying success on thin-needled hosts (Bendall et al., 2017). If host-associated selection on needle width favors differences in ovipositor morphology and there is heritable variation in this trait, populations may exhibit host-associated phenotypic divergence. To control for the potential impact of rearing host on ovipositor morphology (i.e., plasticity), all females were reared on *P. banksiana* for at least two generations prior to dissection. We dissected, mounted, imaged (IMAGEJ v1.51; Schneider et al., 2012), and laid 30 landmarks (Figure 3B) defining the overall shape of ovipositors, from a total of 28 females ($n = 10$ Pitch females, 10 Shortleaf females, 8 Virginia females) as described in Bendall et al. (2017). We then compared the ovipositors from each host using a geometric morphometric analysis, which computes shape differences while controlling for ovipositor size. We aligned the landmarks of each ovipositor using a general Procrustes alignment in GEOMORPH (v4.0.5; Adams & Otárola-Castillo, 2013) implemented in R. Shape differences were visualized via a principle component analysis and assessed for significance using Procrustes ANOVA with sawfly line as a fixed factor. We also assessed differences in ovipositor length and width among sawfly lines using Type II ANOVAs.

Assessment of mechanisms underlying IBE pattern Natural selection against immigrants

To assess whether sawfly lines differ in their performance on different hosts, we reared the offspring of mated, lab-reared females from each host line on each of the three pine species. Briefly, after being mated to a same-host male, each female was placed in a mesh sleeve cage (25.4 cm $\times$ 50.8 cm) with a single seedling of one of the three host plant species (*P. echinata*, *P. virginiana*, or *P. rigida*; “no choice” scenario; see below). For each combination of host species and sawfly line (9 total), we performed 12–29 no-choice assays for a total of 144 no-choice assays.

No-choice cages were checked daily for eggs or until the female died, ultimately yielding between 5 and 10 families for each of the 9 sawfly-line $\times$ host-plant combinations. For each egg-bearing tree, the number of eggs laid was counted. Because *N. lecontei* females are born with their entire complement of eggs and tend to lay this entire complement in a single bout, the number of eggs laid on a tree would represent a female's entire reproductive output. Egg-bearing trees were checked daily and watered as needed until larvae hatched (~2 weeks). Because *N. lecontei* are gregarious and fare poorly when isolated, we reared siblings together in a single rearing box. Larvae were fed clippings from the same host species

they hatched from ab libitum until cocooning. Cocoons were collected as they were spun, watered briefly to promote hardening, and weighed within 48 h of collection.

We assessed performance differences between the lines in three ways: egg-to-cocoon survival, development time to cocoon, and cocoon weight. To determine whether egg-to-cocoon survival rate on different hosts differed among the lines, we examined survival to cocoon at the colony level, where each colony was a family that consisted of a group of siblings with some number of eggs that hatched and survived to the cocoon stage and some number of eggs that did not. We excluded families for which no eggs hatched, as this is often due to external variables (e.g., seedling death). We then used the *glmer* function (lmerTest package) to fit a mixed-effects logistic regression model with a logit link function to the survival data, with colony ID included as a random effect to account for differences among families in hatching success and larval performance unrelated to line of origin. Our model included line, rearing host, and a line-by-host interaction as fixed effects. To evaluate the significance of the two main effects and their interaction, we used a Type III ANOVA. For statistically significant main effects/interaction, post hoc tests were used to assess whether egg-to-cocoon survival was lower in sawflies reared on non-source versus source hosts. For each set of contrasts, we used a Benjamini–Hochberg correction for multiple comparisons.

For the subset of larvae that survived to the cocoon stage, we next asked whether the egg-to-cocoon development time on different hosts differed among the lines. Development time for each cocoon was calculated as the number of days between egg laying (the date on which the female was introduced into the sleeve cage) and cocoon spinning (the date the cocoon was collected in the rearing box, as recorded in our lab rearing logs). We fit a mixed-effects Gamma regression model to the development time data, with colony ID included as a random effect and line, rearing host, and a line-by-host interaction as fixed effects. We evaluated the significance of the main effects and their interaction with a Type III ANOVA and performed post hoc comparisons as described above.

Finally, we asked if the sawflies that spun cocoons differed in weight. For females, which emerge with their full complement of eggs, cocoon weight correlates strongly with fecundity (Harper et al., 2016). For males, body size correlates with reproductive success (Glover et al., 2023). Because cocoon size is sexually dimorphic, we inferred the sex of each cocoon from weights and analyzed male and female cocoons separately. For each sex, we fit a linear mixed model to the individual cocoon weights, with line and host as fixed effects and family as a random effect. We did not include a line $\times$ host interaction in the final models for male and female cocoon weights because the interaction term was not significant. After fitting the models, we evaluated the significance of host and line effects with type II ANOVAs and performed post hoc comparisons as above among lines and hosts for each sex.

Sexual selection against immigrants

To determine whether sawflies from each source host line are more likely to mate with individuals from the same host line, we conducted no-choice mating assays. We chose no-choice assays because one-on-one encounters most closely approximate mating in the wild (females often flee if approached by multiple males in the wild; Benjamin, 1955). For each assay, a single virgin female was placed in a new, plastic 60 mm $\times$ 12 mm petri dish and offered a virgin male from either the

same line (Shortleaf $\times$ Shortleaf, Virginia $\times$ Virginia, and Pitch $\times$ Pitch) or a different line (Shortleaf $\times$ Pitch, Shortleaf $\times$ Virginia, and Virginia $\times$ Pitch, and the reciprocal crosses). To tease apart mating preferences from host preferences (which we evaluate below), we conducted mating assays in the absence of host material. To minimize the impact of inbreeding avoidance (Harper et al., 2016) on same-line mating assays, we obtained males and females from different propagation cages. Sets of six assays (three same lines and three different lines) were recorded for 75 min. We switched the position of same- and different-line pairings in each video to minimize positional biases. A total of 60 assays were performed for each type of pair, with 30 assays in each direction for different-line pairs. For example, to determine whether there was sexual selection against immigrants between Shortleaf (SL) and Virginia (VA) lines, we set up 30 SL $\varnothing$ $\times$ VA σ crosses and 30 VA $\varnothing$ $\times$ SL σ crosses. In total, we recorded 360 no-choice mating assays. After filming, we reviewed the footage and recorded if mating occurred or not. We defined a mating event as an observed copulation lasting at least 60 s (Glover et al., 2023).

To test for differences in mate preference, we used the *glm* function in R with a logit link function to fit a binomial regression model to the mating outcome data, with female source (Shortleaf, Pitch, or Virginia), male source (Shortleaf, Pitch, or Virginia), and female source $\times$ male source interaction term as predictors. To evaluate the significance of the two main effects and their interaction, we used a Type III ANOVA.

Natural selection against hybrids

If natural selection acts against hybrids, offspring that are produced by crosses between different lines should have reduced fitness relative to offspring produced by parents from the same line. Ideally, hybrid performance would be compared to nonhybrid performance in all parental habitats, but for our study, the number of cross $\times$ host combinations was prohibitive (9 possible line combinations $\times$ 3 hosts = 27 treatments). Therefore, as a first step to evaluating the potential for reduced hybrid fitness to generate IBE in this system, we examined egg-to-cocoon survival of hybrids and non-hybrids on a single host, *Pinus banksiana*, not present at our study site. While this approach would not detect some potential sources of selection against hybrids, our rationale for using a non-native host was that we would still be able to detect reduced hybrid performance caused by genetic incompatibilities (e.g., due to physical linkage to or pleiotropic effects of divergently selected host use loci) or by maladaptive trait combinations (e.g., Bendall et al., 2017; Thompson et al., 2021) that cause hybrids to fare poorly on the non-native host.

To produce hybrid and nonhybrid larvae, we used mated females from our sexual isolation assays. After mating was observed, females were released into mesh cages with eight *P. banksiana* seedlings. For each egg-laying female, we recorded the number of eggs laid, reared larvae on *P. banksiana* foliage as described above, and recorded the number of cocoons produced by each family. Due to variation in mating propensities and willingness to lay eggs, this resulted in an uneven number of families for the different crosses (P = Pitch, SL = Shortleaf, VA = Virginia): P $\varnothing$ $\times$ P σ : $n = 9$, P $\varnothing$ $\times$ SL σ : $n = 10$; P $\varnothing$ $\times$ VA σ : $n = 10$; SL $\varnothing$ $\times$ P σ : $n = 4$; SL $\varnothing$ $\times$ SL σ : $n = 10$; SL $\varnothing$ $\times$ VA σ : $n = 1$; VA $\varnothing$ $\times$ P σ : $n = 11$; VA $\varnothing$ $\times$ SL σ : $n = 12$; VA $\varnothing$ $\times$ P σ : $n = 9$.

To test the prediction that hybrid families had reduced survival compared to nonhybrid families, we fit a mixed-effects

logistic regression model with a logit link function to the survival data, with maternal line, paternal line, and their interaction as fixed effects and colony ID as a random effect to account for differences among families in hatching success and larval performance unrelated to line of origin. To evaluate the significance of the two main effects and their interaction, we used a Type III ANOVA.

Dispersal bias via habitat preferences

We evaluated host preferences for all three lines with both no-choice and choice assays. No-choice assays were set up as described above (see “Natural selection against immigrants”). To determine whether lines differed in their willingness to lay eggs, we fit a logistic regression model to the binary (laid or did not lay) no-choice assay outcome data as a function of sawfly line, host plant, and their interaction. We used a Type III ANOVA to evaluate the significance of model terms and performed post hoc tests.

For choice assays, individual females were released singly into 33 cm × 33 cm × 61 cm mesh cages with two seedlings of each of two host species: the source host for the focal female and one of the two alternative host species. For choice assays, we used unmated females, which can lay unfertilized eggs that develop into haploid males and exhibit the same host preferences as mated females (Bendall et al., 2017). Due to constraints on space and availability of adult females and pine seedlings, we were unable to perform choice assays between nonsource host pairs. In total, we conducted 193 choice assays, with roughly equal numbers across 6 experiments: Virginia females, $n = 61$ ($n = 30$ for *P. virginiana* vs. *P. echinata* assays; $n = 31$ for *P. virginiana* vs. *P. rigida* assays); Shortleaf females, $n = 62$ ($n = 31$ for *P. echinata* vs. *P. rigida* assays; $n = 31$ for *P. echinata* vs. *P. virginiana* assays); Pitch females, $n = 70$ ($n = 33$ for *P. rigida* vs. *P. echinata* assays; $n = 37$ for *P. rigida* vs. *P. virginiana* assays). With these data, we asked whether females that laid eggs exhibited a preference for their source pine over alternative hosts in pairwise choice assays. Because each line had a unique set of choice assays, we analyzed each line separately. To determine whether a female from a particular line chose her source pine more often than expected by chance (50% based on equal numbers of source and nonsource hosts offered), we used one-tailed binomial exact tests (*binom.test* function in R). We note that because our host-choice assays were conducted in small, confined arenas—which circumvent any long-range cues females may use to choose oviposition hosts—our assays are likely to underestimate the total effect of divergent habitat preferences on dispersal bias.

Habitat-related differences in phenology

To assess differences in the timing of the reproductive period between host lines, we tracked adult eclosion dates of all colonies collected in the field and returned to the lab in 2013 and 2014. Although *N. lecontei* typically has two to three generations per year in Kentucky, sawfly abundance varies across generations. Therefore, we focused our analyses on colonies that yielded at least 20 adults for the generation which we had sampling data for all three hosts available. In total, we collected eclosion data from seven and six Virginia colonies, five and three Shortleaf colonies, and three and three Pitch colonies in 2013 and 2014, respectively. In each year, we tracked eclosion from the date of the first adult emergence. To

quantify differences in adult phenology, we calculated pairwise estimates of temporal isolation (I) between populations following Feder et al. (1993):

$$1 - \left(\frac{\sum x_i y_i}{\sqrt{\sum x_i^2 \cdot \sum y_i^2}} \right) \cdot 100$$

where x_i and y_i represent the proportion of the total number of live adults from host x or y on day i . We assumed an average lifespan of 5 days for females and 4 days for males based on field estimates (Benjamin, 1955) and our own rearing experience. Following Powell et al. (2014), we assessed patterns of eclosion between source hosts by comparing the shape of cumulative eclosion curves for all adults collected from each of the three host species using bootstrapped Kolmogorov–Smirnov tests with 10,000 bootstraps with the *ks.boot* function from the R module MATCHING (v4.10-8; Sekhon, 2008).

Results

Host plants are morphologically and chemically distinct

The three host plants differed from each other in all characteristics measured. For needle width, we found significant host-species and host-age effects, as well as a significant species-by-age interaction (Figure 2A; Supplementary Figure S2; Supplementary Table S6). For mature trees, all host species differed significantly from each other in needle width, with *P. echinata* having the thinnest needles and *P. rigida* the thickest. Except for *P. echinata* ($p = .73$), all seedlings were significantly thinner than their mature counterparts ($p < .05$).

Pinus virginiana differed from *P. rigida* and *P. echinata* in total volatile profiles (Figure 2B). This finding is consistent with a phylogenomic analysis that revealed *P. rigida* and *P. echinata* are more closely related than either is to *P. virginiana* (Jin et al., 2021). Variation in the volatile ratios appeared to drive the discrimination we observed in the total profile, as all abundant compounds (β -pinene, β -phellandrene, α -pinene, α -phellandrene, camphene, β -caryophyllene) and most minor compounds were produced by all three pines. Ratios of the abundant volatiles with linalool and β -caryophyllene varied by pine species (Supplementary Figure S3). *Pinus rigida* and *P. echinata* emitted different ratios of α -pinene, β -pinene, β -phellandrene, and β -caryophyllene against linalool (Supplementary Figure S3), which is noteworthy because these two species did not resolve based on total volatile profiles alone.

For resin content, there were significant host-species and sampling-month effects, and a significant interaction between host species and month (Figure 2C, Supplementary Table S7). Notably, *P. echinata* had less resin early in the sawfly season (May) than either *P. rigida* or *P. virginiana*. Resin content declines in those hosts later in the season (August), such that no significant differences are observed. *P. rigida* and *P. virginiana* do not differ in resin content in May or August.

Genomic data support IBE, but not historical isolation, among sawflies from different pines

Our ddRAD sequencing yielded 1.89 ± 2.35 (SD) million single-ended reads per individual; of which, 1.88 ± 2.34 million survived quality filtering. After alignment, paralog filtering, and

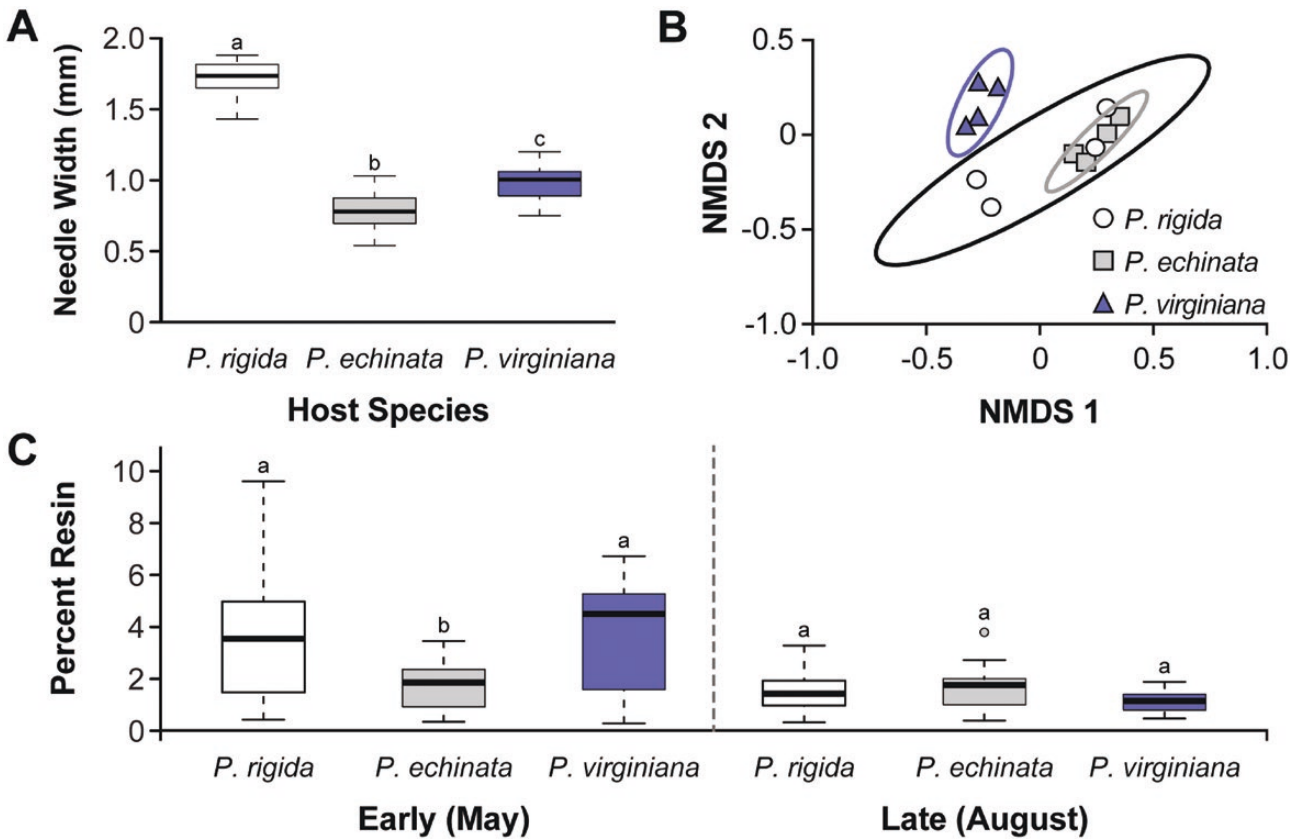


Figure 2. Host plant morphology, volatile profile, and resin content. (A) Needle widths for mature trees sampled at the “Trail of Pines.” (B) NMDS plot representing total volatile blend for the three host species. The volatile blend of *P. virginiana* is distinct, but those of *P. rigida* and *P. echinata* overlap. (C) Resin content variation between the three hosts early (May) and late (August) in the sawfly season. For (A) and (C), boxes represent interquartile ranges (median $\pm$ 2 SD), with outliers indicated as points; different letters represent comparisons that significantly differed in post hoc comparisons.

removal of putative PCR duplicates, an average of 0.95 ± 0.91 million alignments survived and were formed into an average of $15,789 \pm 7,271$ RAD loci per individual with an average coverage of $45.67 \pm 25.15\times$. These loci contained 33,674 SNPs. After removing seven individuals with high levels of missing data, two putatively haploid individuals, and enforcing a $<30\%$ missing data filter, the number of SNPs was reduced to 17,165. After applying the Hardy–Weinberg and minor allele frequency (MAF = 0.05) filters and subsampling to a single SNP per locus, our final data set consisted of 6,759 SNPs.

Using this data set, our evaluation of population structure selected $K = 1$ as the optimal number of clusters across all 100 independent runs, with CV error steadily increasing with K (Supplementary Figure S4). Values of $K > 1$ produced clustering solutions that were both unstable (multiple clustering solutions) and biologically uninterpretable (no clear assignment patterns). Furthermore, investigation of the clustering solutions offered under $K = 2$ and $K = 3$ revealed no meaningful structure between host lines (Supplementary Figures S5 and S6). Overall, genome-wide pairwise F_{ST} between lines was modest (Table 1). Differentiation was significant between the Shortleaf and Virginia lines. Although differentiation between Shortleaf and Pitch was similar to that observed for Virginia and Pitch, permutation tests were not quite significant.

Sawfly lines from different pines differ in ovipositor shape

Ovipositor shape differed among sawfly lines (Figure 3A; Supplementary Table S8). Shortleaf females differed from

Table 1. Pairwise F_{ST} for larvae collected on three different *Pinus* species. Pairwise F_{ST} is shown on the upper diagonal, and p -values are shown on the lower diagonal. Pairwise F_{ST} values were computed using Hudson’s estimator. p -Values were obtained via permutation. Bolded values indicate significant differentiation at $\alpha = .05$.

	Pitch	Shortleaf	Virginia
Pitch	—	$F_{ST} = 0.0132$	$F_{ST} = 0.0047$
Shortleaf	$p = .0571$	—	$F_{ST} = 0.0144$
Virginia	$p = .2706$	$p = .0168$	—

both Virginia and Pitch female, but Virginia and Pitch females did not differ in ovipositor shape. We did not detect differences in ovipositor length or width among sawfly lines (Supplementary Table S9).

Evidence of natural selection against immigrants

For survival to cocoon, there was a significant effect of sawfly line and a significant line-by-rearing host interaction (Figure 4A, Supplementary Table S10). Overall, the Shortleaf line had higher egg-to-cocoon survival than the other two lines. Within the Pitch and Shortleaf lines, we did not detect any survival differences based on rearing host. However, the Virginia line had significantly reduced survival when reared on *P. rigida* compared to *P. virginiana* or *P. echinata*. For development time, all model terms (line, host, host-by-line interaction) were significant (Figure 4B; Supplementary Table S11).

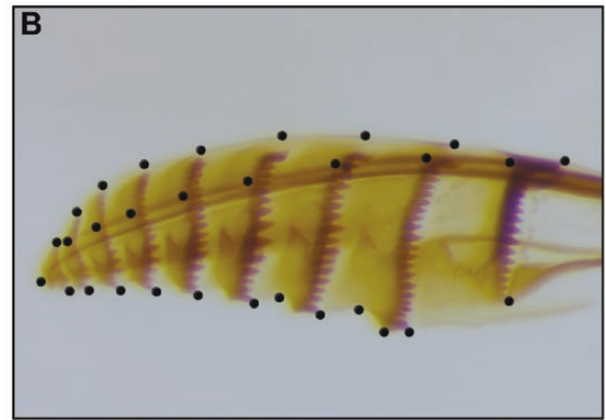
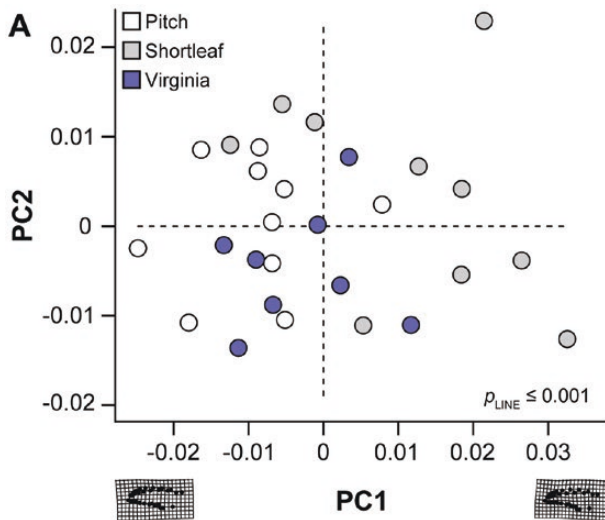


Figure 3. Variation in ovipositor morphology. (A) Principle components analysis of overall ovipositor morphology, with Pitch females shown in white, Shortleaf females shown in gray, and Virginia females shown in blue. The warp grids demonstrate the change in shape along PC1. Shortleaf females have significantly differently shaped ovipositors than Pitch and Virginia females. (B) Image of *Neodiprion lecontei* ovipositor showcasing the landmarks used in the analysis.

Notably, each sawfly line tended to develop fastest when reared on its original source host (i.e., Virginia sawflies developed fastest when reared on *P. virginiana*). Averaged across hosts, the Shortleaf line tended to develop faster than the other two lines. Averaged across lines, sawflies tended to develop slower on *P. rigida* than on the other two hosts. For female cocoon weight, we found significant sawfly-line and rearing-host effects, but their interaction was not significant (Figure 4C, Supplementary Table S12). Overall, Shortleaf females weighed less than Pitch and Virginia females. Additionally, regardless of sawfly line, females reared on *P. echinata* tended to weigh less than females reared on other pines. Male cocoon weight results were qualitatively very similar to those for female cocoon weight (Supplementary Figure S7, Supplementary Table S13): There were significant line and host effects, and Shortleaf males weighed less than males from other lines. One difference, however, was that males reared on *P. virginiana* were significantly heavier than those reared on other hosts. Together, these data indicate that there are substantial differences in larval performance traits within and among sawfly lines and host plants. These data also indicate that possible costs to immigrants (i.e., sawflies that choose a host species that differs from their source host) include reduced survival of eggs to cocoon (Figure 4A: Virginia line) and longer development times (Figure 4B: all lines).

No evidence of sexual selection against immigrants or natural selection against hybrids

Neither female line nor male line had any effect on mating outcomes in no-choice assays (Supplementary Table S14). Likewise, the combination of male and female lines (male $\times$ female interaction) did not affect mating outcome. Together, these results indicate that females and males from all lines were equally willing (or unwilling) to mate, regardless of whether they were paired with an individual that came from the same or a different host species. As all individuals used in this assay were reared on *P. banksiana*, however, we cannot rule out the existence of mate discrimination using a host-odor-related cue in natural populations.

When we reared offspring of mated pairs on a non-native host, we found no effect of maternal line, paternal line, or their interaction on offspring survival to cocooning (Supplementary Table S15). The hybrid offspring produced by the different-host pairings did not have any obvious reduction in survival compared to nonhybrid offspring, although we note that sample sizes for some cross types were small. In addition, there could be other sources of reduced hybrid fitness (e.g., reduced survival on other host species, reduced adult reproductive success) our approach could not detect.

Some evidence of biased dispersal via habitat preferences

In no-choice assays, the host plant offered did not influence a female's likelihood of ovipositing, nor was there a significant line-by-host interaction (Supplementary Table S16). However, there was a significant effect of the female's line on overall willingness to lay eggs (Figure 5A). Specifically, Shortleaf females were more likely to oviposit than Pitch and Virginia females. In choice assays, only Virginia females were significantly more likely to lay their eggs in their host (*P. virginiana*) than a nonsource host (*P. echinata* or *P. rigida*) (Figure 5B; Supplementary Table S17). Although we could not compare choice assays directly because they were set up differently for different lines, we note that the proportions of females from each line that laid eggs in choice assays were very similar to those observed in no-choice assays: 81% of Shortleaf females, 54% of Pitch females, and 61% of Virginia females laid eggs in choice assays. Overall, our host preference assays reveal that Pitch and Virginia females are more reluctant to lay on pine seedlings than Shortleaf females and that Virginia females prefer their source host.

Evidence of host-related phenology differences

Although patterns of eclosion varied between host plants and between years, there was at least partial temporal isolation between the lines in each year (Figure 6; Table 2). In 2013,

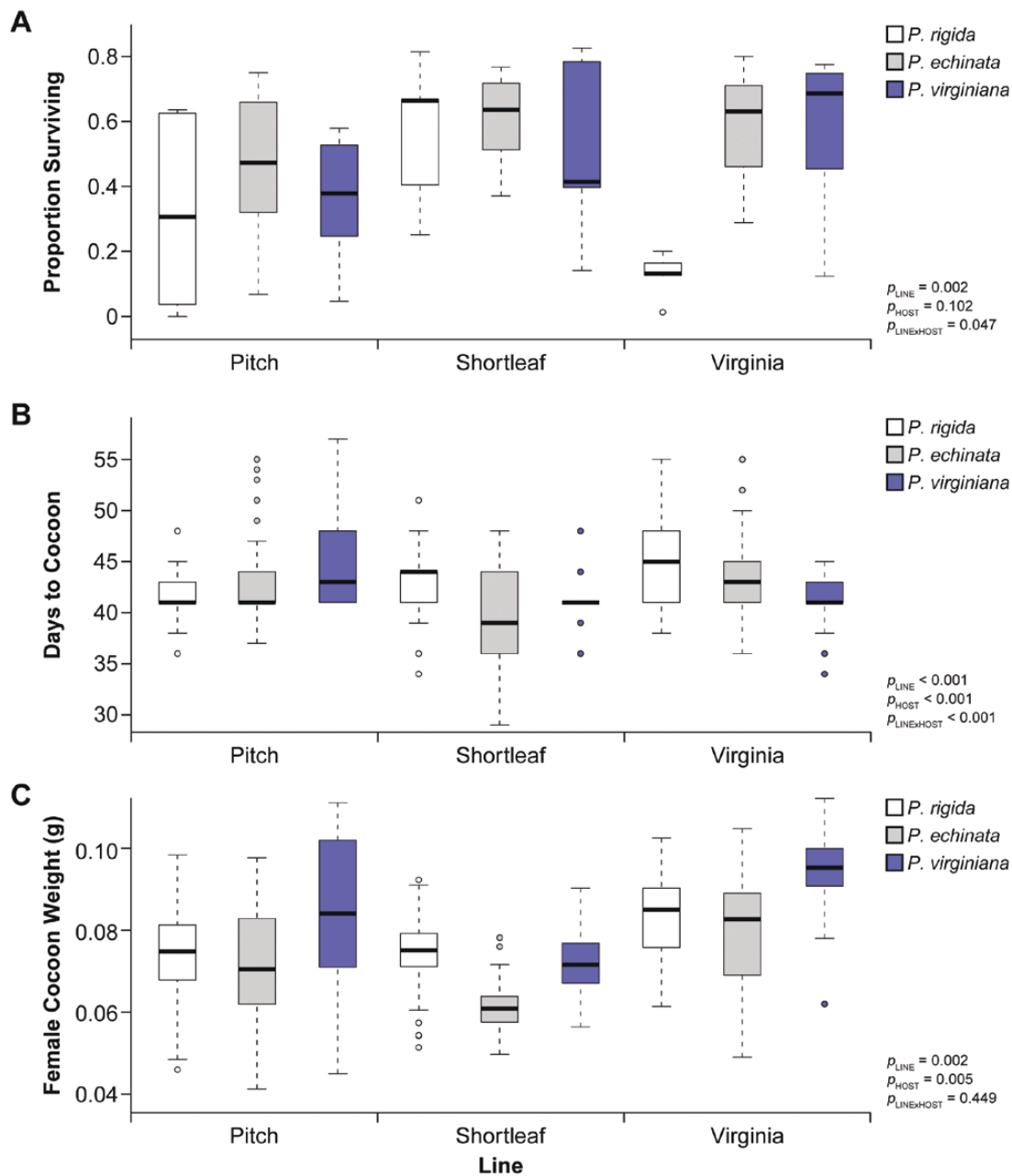


Figure 4. Evidence of selection against immigrants. (A) Proportion of larvae surviving from egg to cocoon stage. (B) Development time from egg to cocoon. Sawflies tended to develop fastest on their source host. (C) Female cocoon weight. Shortleaf females had the lowest cocoon weights, and females reared on *P. echinata* tended to be smaller than those reared on other hosts. For all panels, boxes represent interquartile ranges (median ± 2 SD), with outliers indicated as points. Model statistics are given for each panel, with statistics for post hoc pairwise comparisons given in [Supplementary Tables 10–12](#).

all sawfly lines differed significantly in their eclosion pattern, although the Pitch and Virginia lines were less strongly isolated than the other comparisons. In 2014, the Shortleaf and Virginia lines did not significantly differ in eclosion pattern and were also less isolated than in 2013. Conversely, although their eclosion patterns significantly differed in both years, the Pitch and Virginia lines were more strongly isolated in 2014 than in 2013. The Pitch and Shortleaf lines significantly differed in eclosion patterns and were strongly isolated in both years.

Discussion

In many species, including the redheaded pine sawfly *N. lecontei*, genetic differentiation between populations

increases as their environments become more dissimilar ([Bagley et al., 2017](#); [Sexton et al., 2014](#); [Shafer & Wolf, 2013](#); [Wang et al., 2013](#)). To better understand how a pattern of IBE evolves, we characterized patterns and mechanisms of divergence in a sympatric population of *N. lecontei* that recently colonized three pine hosts. We first characterized differences in needle structure, volatile profiles, and defensive chemistry among the three pine host plants that could generate divergent selection pressures, bias dispersal patterns, and promote phenological differences. Next, we evaluated patterns of genetic differentiation among sawflies collected from the different hosts, finding modest differentiation between the three lines consistent with recent colonization and in situ divergence. Our laboratory assays support three potential

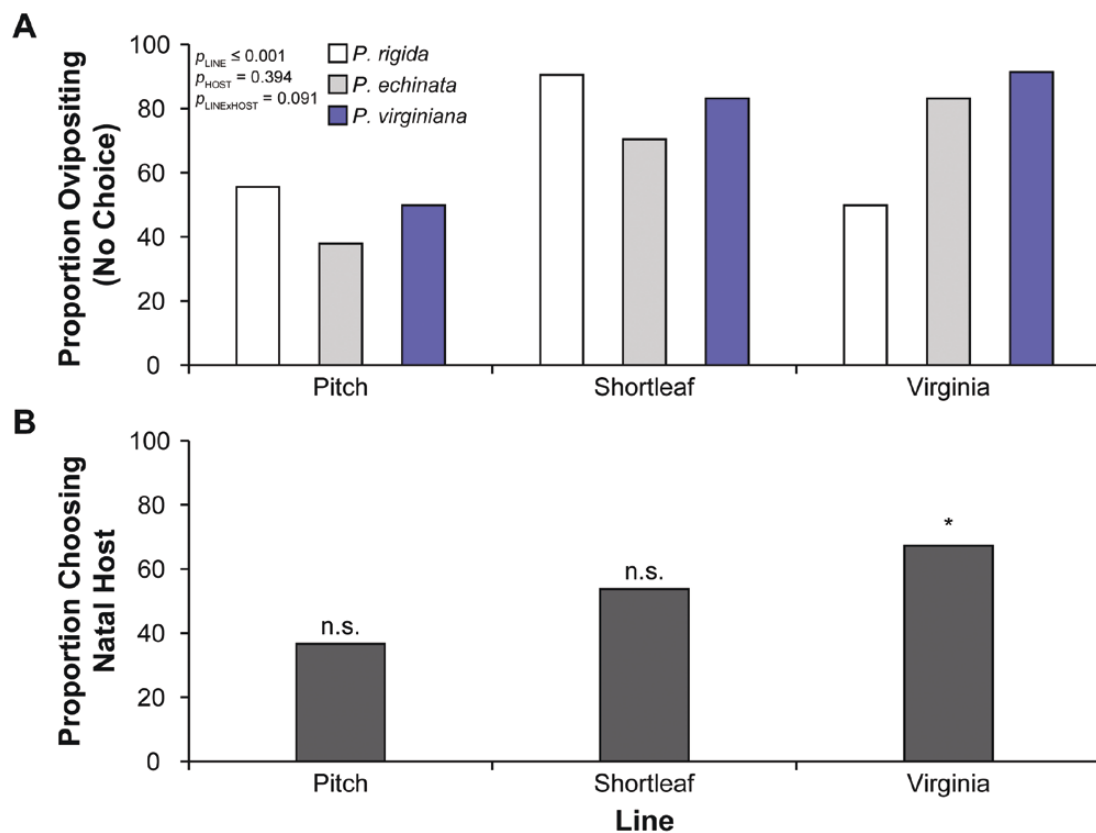


Figure 5. Evidence of dispersal bias amongst sawfly lines. (A) Proportion of females ovipositing on each *Pinus* host in no-choice assays. p -Values for model terms are given; post hoc comparisons among sawfly lines are in [Supplementary Table S16](#). (B) Proportion of females choosing their source host over nonsource hosts in choice assays. Only Virginia females demonstrated a preference for their source host (*P. virginiana*). For (B), ^{n.s.} $p > .05$; * $p < .05$.

mechanisms generating IBE in *N. lecontei*: natural selection against immigrants, biased dispersal, and host-related differences in phenology. However, we find no evidence of sexual selection against immigrants or selection against hybrids in this system. Overall, our results suggest that different mechanisms can contribute to IBE between populations even when they belong to the same species and occupy the same geographic location. Below, we consider limitations of our data and discuss how these results impact our understanding of *Neodiprion* divergence and, more generally, how environmental differences shape patterns of genetic variation in nature.

Emergence of phenotypic differentiation and IBE

Our population structure analyses indicate the sawflies at the “Trail of Pines” form a single genetic cluster, a result that rules out recent colonization of the site by multiple, strongly differentiated lineages. It is therefore possible that the host-associated phenotypic and genetic differentiation we observed arose in situ and rapidly (within <40 generations). In further support of a single-colonization scenario, the three pine species at the “Trail of Pines” site are uncommon in Lexington, KY, with the nearest large host populations ~100 km away. Given this host distribution and the young age of the Trail of Pines *N. lecontei* population, independent colonization by previously diverged sawfly lineages followed by homogenization seems unlikely. Nevertheless, additional data and analysis are needed to formally evaluate alternative demographic scenarios.

Rapid phenotypic adaptation to host plants has been documented in other insect systems (e.g., [Singer et al., 1993](#); [Sousa](#)

[et al., 2019](#); [Thompson, 1998](#)), often following introduction of pest insects or novel host plants. For example, within the past 50–80 years, several host races of the American soapberry bug (*Jadera haematoloma*) have evolved, with the mouthpart (“beak”) length corresponding to the size of the host’s fruit ([Carroll & Boyd, 1992](#); [Comerford et al., 2022](#)). These host races also have faster development time and greater survival on their novel hosts ([Carroll et al., 1997, 1998](#)). In our study population, patterns of host-associated phenotypic differentiation among the three pine hosts involved multiple phenotypic traits, including differences in host preference ([Figure 5](#)), larval development on different host plants ([Figure 4B](#)), body size ([Figure 4C](#)), and ovipositor morphology ([Figure 3A](#)). Although phenotypic differences can result from plastic responses to rearing host (e.g., [Görür, 2003](#); [Pfennig et al., 2010](#)), we measured all traits in individuals that had been reared in a common lab environment and on the same non-source host plant (*P. banksiana*), suggesting the observed phenotypic differences were heritable.

Perhaps the most striking morphological differences were between sawflies that originated from *P. echinata* (shortleaf pine) and sawflies that originated from the other two pine species. Shortleaf females had differently shaped ovipositors ([Figure 3A](#)) and Shortleaf cocoons—which predict adult body size—were smaller than cocoons from Pitch and Virginia lines ([Figure 4C](#)). Notably, although all three host species differ in needle width, *P. echinata* has the thinnest needles ([Figure 2A](#)). Our finding that *N. lecontei* adults from the thinnest-needled host were smaller than adults from other hosts is consistent with recent work demonstrating concordant host needle-width

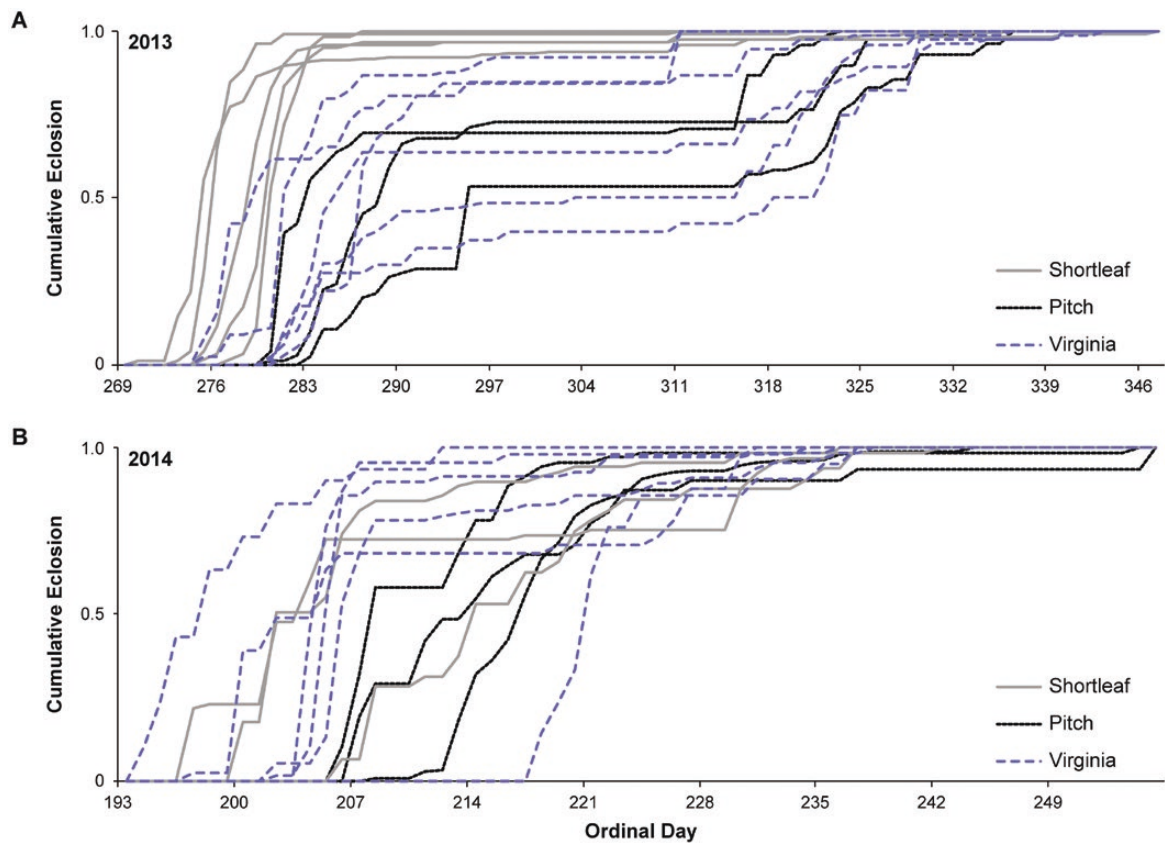


Figure 6. Evidence of host-related phenological differences. Cumulative eclosion curves for individual families with at least 20 eclosing adults from (A) 2013 and (B) 2014. Shortleaf colonies are shown in solid gray lines, Pitch colonies in solid black lines, and Virginia colonies in dashed blue lines. In 2013, all hosts differed significantly in their eclosion patterns. In 2014, Shortleaf and Virginia lines were no longer isolated (see Table 2).

Table 2. Phenological differences in adult eclosion patterns. For each pairwise comparison, the pairwise I values (upper number) and *p*-values from Kolmogorov–Smirnov tests (lower number) are given. Both evaluate differences in the cumulative eclosion curve per source host. Bolded values indicate comparisons where Kolmogorov–Smirnov tests indicate significant differentiation at $\alpha = .05$.

	Pairwise I	
	2013	2014
Pitch × Shortleaf	0.8155 (<i>p</i> < .0001)	0.7026 (<i>p</i> = .01891)
Pitch × Virginia	0.4851 (<i>p</i> = 0.0119)	0.7188 (<i>p</i> = .01891)
Shortleaf × Virginia	0.7004 (<i>p</i> < .0001)	0.3683 (<i>p</i> = .5308)

and adult body-size clines in *N. lecontei* (Glover et al., 2023). Also, our finding that these morphological differences are maintained in sympatry is consistent with experimental work demonstrating that thin-needled pines impose strong selection on ovipositor size and shape (Bendall et al., 2017) and egg size (Glover et al., 2023) in *Neodiprion* females.

In addition to the observed phenotypic differences, we also observed modest genetic differentiation (F_{ST})—or IBE (host)—between Shortleaf lines and the other two lines. However, differentiation between Shortleaf and Pitch lines was not quite significant (Table 1), possibly due to a smaller sample size for this comparison. Given the observed phenotypic

differentiation among the three sawfly lines, the observed genetic differentiation could stem in part from divergent selection on loci encoding traits that impact performance on different pines. A future study incorporating whole-genome, rather than reduced-representation, data would be useful for evaluating signatures of selection across the genome.

Although little is known of the speed at which IBE can emerge, evidence from multiple invasive species indicates that IBD can be generated quickly. For example, IBD was detected within 15 years of invasion amongst Asian tiger mosquitoes collected in villages in the Torres Straight Islands in Australia (Schmidt et al., 2021). The IBD pattern was also detected in the western corn rootworm during both its initial invasion and after establishment and spread through Southern Europe (Lemic et al., 2015). In the pine sawfly family Diprionidae, IBD has been detected in *Diprion similis*, a species that invaded eastern North America in the early 1900s (Davis et al., 2023). Here, we report evidence consistent with the rapid emergence of IBE at a single site, likely within no more than 40 generations. Given the potentially important role that selection plays in generating IBE, it is possible that IBE tends to emerge more quickly than IBD. However, much more work is needed to evaluate the relative speed with which these different patterns of population structure tend to emerge.

Multiple mechanisms contribute to IBE in pine sawflies

Our work supports at least three mechanisms contributing to IBE in pine sawflies. First, our larval performance experiments

suggest there may be natural selection against immigrants on different pines. For example, all lines developed most quickly on their source host (Figure 4B). Under field conditions, prolonged development on alternative hosts could impose selection against immigrants via increased exposure to a large community of pathogens, parasitoids, and predators (see Forbes et al., 2018; Hanski & Parviainen, 1985; Holling, 1959; Olofsson, 1987; Wilson et al., 1992). Natural selection against immigrants was also evident for the Virginia line, which had reduced survival to the cocoon stage when reared on a nonsource host, *P. rigida* (Figure 4A). Finally, although we found no evidence to indicate that sawflies produced larger cocoons when reared on their source host, Shortleaf males and females tended to have smaller cocoons than males and females from the other lines (Figure 4C; Supplementary Figure S7). Although lower cocoon weights are associated with reduced fecundity in females (Harper et al., 2016), reduced body size and egg size are also associated with increased hatching success on thinner needled pines (Bendall et al., 2017; Glover et al., 2023). Thus, smaller body sizes could explain why the Shortleaf line had the highest egg-to-cocoon survival rates in our laboratory assays (Figure 4A), which used seedlings for oviposition hosts. Notably, seedlings have even thinner needles than any of the mature hosts (Supplementary Figure S2).

However, because we did not directly evaluate hatching success in our survival assays, additional experiments are needed to determine the relative contribution of adult female oviposition traits and larval feeding traits to immigrant inviability. To more closely approximate selection in the field, such experiments would ideally use mature host plants for oviposition rather than seedlings. More generally, because laboratory assays can miss important sources of divergent selection and immigrant inviability (Hatfield & Schluter, 1999; Kimball et al., 2008; Rundle & Nosil, 2005), field-based diet manipulations are a high priority for future work.

Second, we also found evidence of a potential dispersal bias: in both choice and no-choice assays, Virginia females were more likely to oviposit on *P. virginiana* than on other hosts (Figure 5B). Interestingly, *P. virginiana* has the most distinct volatile profile of the three hosts at the Arboretum (Figure 2B), offering a potential explanation for why the Virginia line was the only sawfly line to demonstrate a strong host preference. Additionally, females from the Virginia line were especially reluctant to oviposit on *P. rigida* (Figure 5A), a host on which this line had reduced survival (Figure 4A). These observations suggest that divergent host preferences in the Virginia line evolved via natural selection. Because *Neodiprion* sawflies mate on their host plant (Benjamin, 1955; Coppel & Benjamin, 1965; Knerer, 1984), divergent habitat preferences also have the potential to reduce gene exchange among hosts. Host fidelity—the tendency of individuals to reproduce on their natal host type—has long been thought to facilitate sympatric speciation (Feder et al., 1994; Hirai et al., 2006; Wood et al., 1999). Similarly, the reduced gene flow that results from divergent habitat preferences may frequently contribute to IBE.

Third, across 3 years, we found evidence of strong, but sometimes variable temporal isolation among the three hosts (Figure 6; Table 2). Overall, Pitch and Shortleaf lines were the most consistently and strongly isolated in terms of phenology, with Shortleaf adults tending to emerge early and Pitch adults tending to emerge late. This partial temporal isolation

is likely to reduce gene exchange, enabling neutral regions of the genome to diverge via drift. Like host fidelity, temporal isolation is thought to play an important role in promoting speciation in the absence of geographic barriers (reviewed in Taylor & Friesen, 2017). Not only is temporal isolation a particularly effective barrier to gene exchange (Abbot & Withgott, 2004; Feder et al., 1993, 1994), variation in abiotic and biotic selection pressures among habitats often generate divergent selection on reproductive timing (Burban et al., 2020; Hood et al., 2019; Santos et al., 2011; Svensson et al., 2005; Thomas et al., 2003). For these reasons, temporal isolation may be an especially common mechanism generating IBE.

Although temporal isolation appears to contribute to IBE in *N. lecontei*, we cannot determine from our data—which were collected from field-caught mid-late-instar larvae reared to adulthood in the lab—whether variation in adult emergence times is due to genetic variation, rearing environment, or both. One potential explanation for differences in adult emergence timing is that these differences evolved via natural selection to optimize timing for different hosts (e.g., Feder & Forbes, 2010; Feder et al., 1993, 1994). Although we do not yet know which host cues *N. lecontei* females use when selecting host plants for oviposition (but see Björkman et al., 1997; Tisdale & Wagner, 1991), we do know that pines as a whole (Nerg et al., 1994) and the specific trees at the Trail of Pines (Figure 2C) vary seasonally in resin content. Pines also vary seasonally in moisture levels (C. E. Van Wagner, 1967) and volatile profile (Geron & Arnts, 2010). If the seasonal variation in host quality differs between the three host species, selection could favor different peak emergence times among sawflies using different pines. Finer grained analysis of seasonal variation in host quality, impact on sawfly reproductive success, and heritability of adult emergence times are required to evaluate this hypothesis. Eclosion differences could also be generated via plasticity in development time. Developmental plasticity is well documented in insects (see Nylin & Gotthard, 2003), with many examples demonstrating that larvae develop at different rates when reared on different diets. Indeed, our results indicate that rearing host affects developmental timing, with evidence of a genotype-by-host interaction as well (Figure 4B). Confirming that differences in the speed of egg-to-cocoon development give rise to differences in adult eclosion timing in the field will require additional field surveys.

Unlike immigrant inviability, biased dispersal, and temporal isolation, we did not find any evidence of sexual selection against immigrants: Mating outcomes did not differ between same-host and different-host pairs in no-choice mating assays (Supplementary Table S14). By contrast, a recent experiment using the same mating assay design revealed sexual isolation between *N. lecontei* and sister species *N. pinetum*, largely stemming from strong size-based assortative mating within and between species (Glover et al., 2023). Despite some size differences among the three lines in this experiment (Figure 4C; Supplementary Figure S7), these differences were apparently insufficient to produce strong assortative mating by host in our assays. One limitation of our mating assays, however, is that all individuals were reared on the same host plant, potentially minimizing size differences arising because of rearing host. Rearing host clearly influences body size in *N. lecontei* (Figure 4C; Supplementary Figure S7), and work in other insect systems demonstrates that dietary influences on body

size can affect mating outcomes (Forister & Scholl, 2012). Rearing diet could also influence assortative mating via affecting adult pheromone composition, cuticular hydrocarbon profiles, or chemical-based mating preferences (e.g., Conner et al., 1990; Darragh et al., 2019; Gosden & Chenoweth, 2011). Finally, in nature, mating typically takes place on the host plant, so it's possible that the presence of host material interacts with other behavioral and chemosensory cues to influence mating outcomes (e.g., Liao et al., 2016; Sattman & Cocroft, 2003). Thus, to rule out sexual selection against immigrants, additional experiments are needed.

We also did not find evidence of reduced hybrid viability in our assays, as might be expected if there were partial intrinsic postzygotic isolation between the three lines. We note, however, that we did not measure fertility, fecundity, or mating success of hybrids. Also, perhaps the biggest limitation of our hybrid assays was that we did not evaluate host-based sources of reduced hybrid fitness. As ecologically dependent selection against hybrids has been noted in many other insect systems (Servedio, 2004; e.g., *Rhagoletis* flies [Linn et al., 2004], *Timema* walking sticks [Sandoval, 1994a, 1994b], and pea aphids [Via et al., 2000]), it may contribute to IBE in *N. lecontei*. For example, hybrid females between *N. lecontei* and its sister species *N. pinetum* have mismatched host preferences and egg-laying traits that drastically reduce oviposition success (Bendall et al., 2017). Although the magnitude of differences in host preference and ovipositor differences revealed in this study are modest compared to interspecific differences, mismatches in these traits may nevertheless offer at least one mechanism by which hybrid females at the site could have reduced fitness compared to non-hybrids.

It is also notable that these subtle phenotypic differences were sufficient to generate partial reproductive isolation in sympatry. The feasibility of sympatric speciation—or more generally speciation-with-gene flow—is now largely accepted (Berlocher & Feder, 2002; Bolnick & Fitzpatrick, 2007; Hey, 2006; Nosil, 2008; Pinho & Hey, 2010; Servedio & Noor, 2003; Smadja & Butlin, 2011; Via, 2001), with examples found across the tree of life (e.g., Bush, 1975a; b; Martin et al., 2013; Morales et al., 2017; Papadopoulos et al., 2011; Potkamp & Fransen, 2019). Despite this, it is unknown how frequently this speciation mode occurs in nature (Bolnick & Fitzpatrick, 2007). Our results suggest that conditions conducive to divergence with gene flow may be common, at least in host-specialized plant-feeding insects. Although distinct regional host preferences are documented within *N. lecontei* (Benjamin, 1955), it is not uncommon to find sites like the Arboretum where sawflies use multiple host plants (R.K.B. and C.R.L., personal observation). Additional experiments at other sites harboring sympatric populations of *N. lecontei*—as well as in other systems—will be valuable for determining how often using multiple hosts initiates divergence with gene flow and characterizing factors that predict how far this divergence proceeds toward stronger reproductive isolation and, ultimately, ecological speciation (Elias et al., 2012; Hendry, 2009; Thibert-Plante & Hendry, 2011).

Conclusion

Like many taxa investigated to date (Gray et al., 2014; Prunier et al., 2017; Sexton et al., 2014; Shafer & Wolf, 2013; Weber et al., 2017), genetic differentiation among populations of *N. lecontei* correlates with both geographic distance (IBD) and host use (IBE). Here, we take advantage

of a sympatric population of *N. lecontei* on three hosts to explore potential mechanisms producing IBE. Our analyses reveal both that a pattern of IBE could emerge rapidly—in our case, in tens of generations—and that multiple mechanisms, including immigrant inviability, dispersal bias, and temporal isolation, are likely important to generating IBE. These two observations may be related: Perhaps IBE emerges quickly precisely because there are multiple mechanisms simultaneously reducing the effective migration rate between habitats. Still, much work remains to better understand IBE in this system. Additionally, comparative studies across diverse taxa are needed to evaluate the relative importance of different IBE mechanisms, to test the hypothesis that IBE evolves more quickly than IBD, and to determine how often and under what conditions IBE proceeds to ecological speciation. While such work is labor intensive, it is essential for better understanding patterns of genetic variation in nature.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All data sets, input files, R codes, and R scripts used in genetic (F_{ST} calculation and ADMIXTURE population structure analysis), host plant characterization (needle width, volatile profile, and resin content), morphology (ovipositor shape), and IBE assays (natural selection against immigrants, sexual selection against immigrants, hybrid survival, dispersal bias) described in the study are available in standard file formats on DRYAD (data files; <https://doi.org/10.5061/dryad.gf1vhhmvd>) and Zenodo (scripts; <https://doi.org/10.5281/zenodo.8145433>). Short read data are available on the NCBI Short Read Archive (accession numbers SAMN23893938–SAMN23893939, SAMN23893946–SAMN238951, SAMN23893955–SAMN23893956, SAMN23893965, SAMN23893972, SAMN23893997–SAMN23893999, SAMN25157047–SAMN25157050, SAMN25157069, SAMN25157076–SAMN25157077, and SAMN36304574–SAMN36304609).

Author contributions

R.K.B., C.J.F., and C.R.L. designed the research. R.K.B., M.N.H., J.F., J.W., J.W.T., C.J.F., and C.R.L. performed the research. R.K.B., C.J.F., and C.R.L. analyzed the data. R.K.B., J.W.T., C.J.F., and C.R.L. wrote the manuscript with input from all authors.

Funding

This work was supported by the National Science Foundation (DEB-1257739 and DEB-CAREER-1750946 to C.R.L., IOS-2101059 and IOS-1656625 to C.J.F.) and USDA-NIFA (pre-doctoral-fellowship 2015-67011-22803 to R.K.B.), the BIO5 Institute (to C.J.F.), and the University of Kentucky's Ribble Travel Grant (to J.W.T.). For computing resources, we thank the University of Kentucky Center for Computational Sciences and the Lipscomb High Performance Computing Cluster.

Conflict of interest: Editorial processing of the manuscript was done independently of C.R.L., who is an associate editor of *Evolution*. The other authors declare no conflict of interest.

Acknowledgments

We thank the staff of the University of Kentucky's Arboretum and State Botanical Gardens, especially Todd Rounsaville, for allowing us to collect sawflies, clip branches, and conduct research at the site. We also thank the members of the Linnen lab for their assistance with monitoring the site and rearing of pine sawflies used in these experiments. We thank Dylan P'Simer for assistance with the resin extraction protocol. We also thank Vitor Sousa for helpful discussions of F_{ST} analyses, as well as for providing several R scripts.

References

- Abbot, P., & Withgott, J. H. (2004). Phylogenetic and molecular evidence for allochronic speciation in gall-forming aphids (*Pemphigus*). *Evolution*, 58(3), 539–553. <https://doi.org/10.1111/J.0014-3820.2004.TB01677.X>
- Adams, D. C., & Otárola-Castillo, E. (2013). Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, 4(4), 393–399. <https://doi.org/10.1111/2041-210X.12035>
- Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, 19(9), 1655–1664. <https://doi.org/10.1101/gr.094052.109>
- Alexander, R. D., & Bigelow, R. S. (1960). Allochronic speciation in field crickets, and a new species, *Acheta veletis*. *Evolution*, 14(3), 334–346. <https://doi.org/10.2307/2405976>
- Bagley, R. K., Hurst, M. N., Frederick, J., Wolfe, J., Terbot, J. W., Frost, C. J., & Linnen, C. R. (2023). Data from: Multiple mechanisms contribute to isolation by environment in the redheaded pine sawfly, *Neodiprion lecontei*. *Dryad*. <https://doi.org/10.5061/dryad.gf1vhmvd>
- Bagley, R. K., Sousa, V. C., Niemiller, M. L., & Linnen, C. R. (2017). History, geography and host use shape genomewide patterns of genetic variation in the redheaded pine sawfly (*Neodiprion lecontei*). *Molecular Ecology*, 26(4), 1022–1044. <https://doi.org/10.1111/mec.13972>
- Baguette, M., & Van Dyck, H. (2007). Landscape connectivity and animal behavior: Functional grain as a key determinant for dispersal. *Landscape Ecology*, 22(8), 1117–1129. <https://doi.org/10.1007/s10980-007-9108-4>
- Battaglia, L. L., Pritchett, D. W., & Minchin, P. R. (2008). Evaluating dispersal limitation in passive bottomland forest restoration. *Restoration Ecology*, 16(3), 417–424. <https://doi.org/10.1111/j.1526-100X.2007.00319.x>
- Benard, M. F., & McCauley, S. J. (2008). Integrating across life-history stages: Consequences of natal habitat effects on dispersal. *American Naturalist*, 171(5), 553–567. <https://doi.org/10.1086/587072>
- Bendall, E. E., Bagley, R. K., Sousa, V. C., & Linnen, C. R. (2022). Faster-haplodiploid evolution under divergence-with-gene-flow: Simulations and empirical data from pine-feeding hymenopterans. *Molecular Ecology*, 31(8), 2348–2366. <https://doi.org/10.1111/mec.16410>
- Bendall, E. E., Vertacnik, K. L., & Linnen, C. R. (2017). Oviposition traits generate extrinsic postzygotic isolation between two pine sawfly species. *BMC Evolutionary Biology*, 17(1), 26. <https://doi.org/10.1186/s12862-017-0872-8>
- Benjamin, D. M. (1955). *The biology and ecology of the red-headed pine sawfly* (Technical Bulletin 1118, p. 57). United States Department of Agriculture.
- Berlacher, S. H., & Feder, J. L. (2002). Sympatric speciation in phytophagous insects: Moving beyond controversy? *Annual Review of Entomology*, 47, 773–815. <https://doi.org/10.1146/annurev.ento.47.091201.145312>
- Bhatia, G., Patterson, N., Sankararaman, S., & Price, A. L. (2013). Estimating and interpreting FST: The impact of rare variants. *Genome Research*, 23(9), 1514–1521. <https://doi.org/10.1101/gr.154831.113>
- Björkman, C., Larsson, S., Bommarco, R., & Björkman, C. (1997). Oviposition preferences in pine sawflies: A trade-off between larval growth and Defence against natural enemies. *Oikos*, 79(1), 45. <https://doi.org/10.2307/3546088>
- Bolnick, D. I., & Fitzpatrick, B. M. (2007). Sympatric speciation: Models and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, 38(1), 459–487. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095804>
- Bolnick, D. I., & Otto, S. P. (2013). The magnitude of local adaptation under genotype-dependent dispersal. *Ecology and Evolution*, 3(14), 4722–4735. <https://doi.org/10.1002/ece3.850>
- Botta, F., Eriksen, C., Fontaine, M. C., & Guillot, G. (2015). Enhanced computational methods for quantifying the effect of geographic and environmental isolation on genetic differentiation. *Methods in Ecology and Evolution*, 6(11), 1270–1277. <https://doi.org/10.1111/2041-210X.12424>
- Boughman, J. W. (2001). Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, 411(6840), 944–948. <https://doi.org/10.1038/35082064>
- Boumans, L., Hogner, S., Brittain, J., & Johnsen, A. (2017). Ecological speciation by temporal isolation in a population of the stonefly *Leuctra hippopus* (Plecoptera, Leuctridae). *Ecology and Evolution*, 7(5), 1635–1649. <https://doi.org/10.1002/ece3.2638>
- Bowler, D. E., & Benton, T. G. (2005). Causes and consequences of animal dispersal strategies: Relating individual behaviour to spatial dynamics. *Biological Reviews*, 80(2), 205–225. <https://doi.org/10.1017/s1464793104006645>
- Bradburd, G. S., Ralph, P. L., & Coop, G. M. (2013). Disentangling the effects of geographic and ecological isolation on genetic differentiation. *Evolution*, 67(11), 3258–3273. <https://doi.org/10.1111/evo.12193>
- Bricchi, I., Leitner, M., Foti, M., Mithöfer, A., Boland, W., & Maffei, M. E. (2010). Robotic mechanical wounding (MecWorm) versus herbivore-induced responses: Early signaling and volatile emission in Lima bean (*Phaseolus lunatus* L.). *Planta*, 232(3), 719–729. <https://doi.org/10.1007/s00425-010-1203-0>
- Burban, C., Rocha, S., Leblois, R., Rossi, J. P., Sauné, L., Branco, M., & Kerdelhué, C. (2020). From sympatry to parapatry: A rapid change in the spatial context of incipient allochronic speciation. *Evolutionary Ecology*, 34(1), 101–121. <https://doi.org/10.1007/s10682-019-10021-4>
- Burford Reiskind, M. O., Coyle, K., Daniels, H. V., Labadie, P., Reiskind, M. H., Roberts, N. B., Roberts, R. B., Schaff, J., & Vargo, E. L. (2016). Development of a universal double-digest RAD sequencing approach for a group of nonmodel, ecologically and economically important insect and fish taxa. *Molecular Ecology Resources*, 16(6), 1303–1314. <https://doi.org/10.1111/1755-0998.12527>
- Bush, G. L. (1975a). Modes of animal speciation. *Annual Review of Ecology and Systematics*, 6(1), 339–364. <https://doi.org/10.1146/annurev.es.06.110175.002011>
- Bush, G. L. (1975b). Sympatric speciation in phytophagous parasitic insects. In P. W. Price (Ed.), *Evolutionary strategies of parasitic insects and mites* (pp. 187–206). Plenum Press. https://doi.org/10.1007/978-1-4615-8732-3_9
- Butlin, R. K., & Smadja, C. M. (2018). Coupling, reinforcement, and speciation. *American Naturalist*, 191(2), 155–172. <https://doi.org/10.1086/695136>
- Carroll, S. P., & Boyd, C. (1992). Host race radiation in the soapberry bug: Natural history with the history. *Evolution*, 46(4), 1052–1069. <https://doi.org/10.1111/j.1558-5646.1992.tb00619.x>
- Carroll, S. P., Dingle, H., & Klassen, S. P. (1997). Genetic differentiation of fitness-associated traits among rapidly evolving populations of the soapberry bug. *Evolution*, 51(4), 1182–1188. <https://doi.org/10.1111/j.1558-5646.1997.tb03966.x>
- Carroll, S. P., Klassen, S. P., & Dingle, H. (1998). Rapidly evolving adaptations to host ecology and nutrition in the soapberry bug. *Evolutionary Ecology*, 12(8), 955–968. <https://doi.org/10.1023/a:1006568206413>
- Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics.

- Molecular Ecology*, 22(11), 3124–3140. <https://doi.org/10.1111/mec.12354>
- Chen, B., Cole, J. W., & Grond-Ginsbach, C. (2017). Departure from Hardy–Weinberg equilibrium and genotyping error. *Frontiers in Genetics*, 8, 167. <https://doi.org/10.3389/fgene.2017.00167>
- Chhina, A. K., Thompson, K. A., & Schluter, D. (2022). Adaptive divergence and the evolution of hybrid trait mismatch in threespine stickleback. *Evolution Letters*, 6(1), 34–45. <https://doi.org/10.1002/evl3.264>
- Clobert, J., Le Galliard, J. -F., Cote, J., Meylan, S., & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*, 12(3), 197–209. <https://doi.org/10.1111/j.1461-0248.2008.01267.x>
- Comerford, M. S., Carroll, S. P., & Egan, S. P. (2022). A test of adaptive divergence in a newly discovered host association of the soapberry bug *Jadera haematoloma* on Mexican buckeye, *Ungnadia speciosa*. *Entomologia Experimentalis et Applicata*, 170(1), 64–78. <https://doi.org/10.1111/EEA.13118>
- Conner, W. E., Roach, B., Benedict, E., Meinwald, J., & Eisner, T. (1990). Courtship pheromone production and body size as correlates of larval diet in males of the arctiid moth, *Utetheisa ornatrix*. *Journal of Chemical Ecology*, 16(2), 543–552. <https://doi.org/10.1007/BF01021785>
- Coppel, H. C., & Benjamin, D. M. (1965). Bionomics of the Nearctic pine-feeding Dipteronids. *Annual Review of Entomology*, 10(1), 69–96. <https://doi.org/10.1146/annurev.en.10.010165.000441>
- Crispo, E., Bentzen, P., Reznick, D. N., Kinnison, M. T., & Hendry, A. P. (2006). The relative influence of natural selection and geography on gene flow in guppies. *Molecular Ecology*, 15(1), 49–62. <https://doi.org/10.1111/j.1365-294X.2005.02764.x>
- Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R.; 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, 27(15), 2156–2158. <https://doi.org/10.1093/bioinformatics/btr330>
- Darragh, K., Byers, K. J. R. P., Merrill, R. M., McMillan, W. O., Schulz, S., & Jiggins, C. D. (2019). Male pheromone composition depends on larval but not adult diet in *Heliconius melpomene*. *Ecological Entomology*, 44(3), 397–405. <https://doi.org/10.1111/een.12716>
- Davis, J. M., & Stamps, J. A. (2004). The effect of natal experience on habitat preferences. *Trends in Ecology and Evolution*, 19(8), 411–416. <https://doi.org/10.1016/j.tree.2004.04.006>
- Davis, J. S., Sim, S., Geib, S., Scheffler, B., & Linnen, C. R. (2023). Whole-genome resequencing data support a single introduction of the invasive white pine sawfly, *Diprion similis*. *Journal of Heredity*, 114(3), 246–258. <https://doi.org/10.1093/jhered/esad012>
- Doellman, M. M., Egan, S. P., Ragland, G. J., Meyers, P. J., Hood, G. R., Powell, T. H. Q., Lazorchak, P., Hahn, D. A., Berlocher, S. H., Nosil, P., & Feder, J. L. (2018). Standing geographic variation in eclosion time and the genomics of host race formation in *Rhagoletis pomonella* fruit flies. *Ecology and Evolution*, 9(1), 393–409. <https://doi.org/10.1002/ece3.4758>
- Driscoll, A. L., Nice, C. C., Busbee, R. W., Hood, G. R., Egan, S. P., & Ott, J. R. (2019). Host plant associations and geography interact to shape diversification in a specialist insect herbivore. *Molecular Ecology*, 28(18), 4197–4211. <https://doi.org/10.1111/mec.15220>
- Edelaar, P., Siepielski, A. M., & Clobert, J. (2008). Matching habitat choice causes directed gene flow: A neglected dimension in evolution and ecology. *Evolution*, 62(10), 2462–2472. <https://doi.org/10.1111/j.1558-5646.2008.00459.x>
- Elias, M., Faria, R., Gompert, Z., & Hendry, A. P. (2012). Factors influencing progress towards ecological speciation. *International Journal of Ecology*, 2012, 235010. <https://doi.org/10.1155/2012/235010>
- Etges, W. J., & Tripodi, A. D. (2008). Premating isolation is determined by larval rearing substrates in cactophilic *Drosophila mojavensis*. VIII. Mating success mediated by epicuticular hydrocarbons within and between isolated populations. *Journal of Evolutionary Biology*, 21(6), 1641–1652. <https://doi.org/10.1111/j.1420-9101.2008.01601.x>
- Feder, J. L., & Forbes, A. A. (2007). Habitat avoidance and speciation for phytophagous insect specialists. *Functional Ecology*, 21(3), 585–597. <https://doi.org/10.1111/j.1365-2435.2007.01232.x>
- Feder, J. L., & Forbes, A. A. (2010). Sequential speciation and the diversity of parasitic insects. *Ecological Entomology*, 35, 67–76. <https://doi.org/10.1111/j.1365-2311.2009.01144.x>
- Feder, J. L., Hunt, T. A., & Bush, L. (1993). The effects of climate, host plant phenology and host fidelity on the genetics of apple and hawthorn infesting races of *Rhagoletis pomonella*. *Entomologia Experimentalis et Applicata*, 69(2), 117–135. <https://doi.org/10.1111/j.1570-7458.1993.tb01735.x>
- Feder, J. L., Opp, S. B., Wlazlo, B., Reynolds, K., Go, W., & Spisak, S. (1994). Host fidelity is an effective premating barrier between sympatric races of the apple maggot fly. *Proceedings of the National Academy of Sciences of the United States of America*, 91(17), 7990–7994. <https://doi.org/10.1073/pnas.91.17.7990>
- Forbes, A. A., Bagley, R. K., Beer, M. A., Hippee, A. C., & Widmayer, H. A. (2018). Quantifying the unquantifiable: Why Hymenoptera, not Coleoptera, is the most speciose animal order. *BMC Ecology*, 18(1), 21. <https://doi.org/10.1186/s12898-018-0176-x>
- Forbes, A. A., Devine, S. N., Hippee, A. C., Tvedte, E. S., Ward, A. K. G., Widmayer, H. A., & Wilson, C. J. (2017). Revisiting the particular role of host shifts in initiating insect speciation. *Evolution*, 71(5), 1126–1137. <https://doi.org/10.1111/evo.13164>
- Forister, M. L., & Scholl, C. F. (2012). Use of an exotic host plant affects mate choice in an insect herbivore. *American Naturalist*, 179(6), 805–810. <https://doi.org/10.1086/665647>
- Fox, J., & Weisberg, S. (2019). *An R companion to applied regression* (3rd ed.). Sage.
- Frost, C. J. (2023). Overlaps and trade-offs in the diversity and inducibility of volatile chemical profiles among diverse sympatric neotropical canopy trees. *Plant, Cell & Environment*. In press. <https://doi.org/10.1111/PCE.14594>
- Frost, C. J., Appel, H. M., Carlson, J. E., De Moraes, C. M., Mescher, M. C., & Schultz, J. C. (2007). Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters*, 10(6), 490–498. <https://doi.org/10.1111/j.1461-0248.2007.01043.x>
- Frost, C. J., Nyamdari, B., Tsai, C. J., & Harding, S. A. (2012). The Tonoplast-Localized Sucrose Transporter in *Populus* (PtaSUT4) regulates whole-plant water relations, responses to water stress, and photosynthesis. *PLoS One*, 7(8), e44467. <https://doi.org/10.1371/journal.pone.0044467>
- Gavrilets, S., & Vose, A. (2007). Case studies and mathematical models of ecological speciation. 2. Palms on an oceanic island. *Molecular Ecology*, 16(14), 2910–2921. <https://doi.org/10.1111/j.1365-294X.2007.03304.x>
- Geron, C. D., & Arnts, R. R. (2010). Seasonal monoterpene and sesquiterpene emissions from *Pinus taeda* and *Pinus virginiana*. *Atmospheric Environment*, 44(34), 4240–4251. <https://doi.org/10.1016/j.atmosenv.2010.06.054>
- Ghent, A. W., & Wallace, D. R. (1958). Oviposition behavior of the swain jack-pine sawfly. *Forest Science*, 4(3), 264–272. <https://cfs.nrcan.gc.ca/publications?id=36872>
- Glover, A. N., Bendall, E. E., Terbot, J. W., Payne, N., Webb, A., Filbeck, A., Norman, G., & Linnen, C. R. (2023). Body size as a magic trait in two plant-feeding insect species. *Evolution*, 77(2), 437–453. <https://doi.org/10.1093/evolut/qpac053>
- Görür, G. (2003). Phenotypic plasticity of morphological characters in cabbage aphid reared on both radish and cabbage. *Italian Journal of Zoology*, 70(4), 301–303. <https://doi.org/10.1080/11250000309356533>
- Gosden, T. P., & Chenoweth, S. F. (2011). On the evolution of heightened condition dependence of male sexual displays. *Journal of Evolutionary Biology*, 24(3), 685–692. <https://doi.org/10.1111/j.1420-9101.2010.02205.x>
- Gray, M. M., St. Amand, P., Bello, N. M., Galliard, M. B., Knapp, M., Garrett, K. A., Morgan, T. J., Baer, S. G., Maricle, B. R., Akhunov, E.

- D., & Johnson, L. C. (2014). Ecotypes of an ecologically dominant prairie grass (*Andropogon gerardii*) exhibit genetic divergence across the U.S. Midwest grasslands' environmental gradient. *Molecular Ecology*, 23(24), 6011–6028. <https://doi.org/10.1111/mec.12993>
- Hanski, I., & Parviainen, P. (1985). Cocoon predation by small mammals, and pine sawfly population dynamics. *Oikos*, 45(1), 125. <https://doi.org/10.2307/3565230>
- Harper, K. E., Bagley, R. K., Thompson, K. L., & Linnen, C. R. (2016). Complementary sex determination, inbreeding depression and inbreeding avoidance in a gregarious sawfly. *Heredity*, 117(5), 326–335. <https://doi.org/10.1038/hdy.2016.46>
- Hatfield, T., & Schluter, D. (1999). Ecological speciation in sticklebacks: Environment-dependent hybrid fitness. *Evolution*, 53(3), 866–873. <https://doi.org/10.1111/j.1558-5646.1999.tb05380.x>
- Hendry, A. P. (2009). Ecological speciation! Or the lack thereof? *Canadian Journal of Fisheries and Aquatic Science*, 66(8), 1383–1398. <https://doi.org/10.1139/F09-074>
- Hey, J. (2006). Recent advances in assessing gene flow between diverging populations and species. *Current Opinion in Genetics & Development*, 16(6), 592–596. <https://doi.org/10.1016/j.gde.2006.10.005>
- Hirai, Y., Kobayashi, H., Koizumi, T., & Katakura, H. (2006). Field-cage experiments on host fidelity in a pair of sympatric phytophagous ladybird beetles. *Entomologia Experimentalis et Applicata*, 118(2), 129–135. <https://doi.org/10.1111/j.1570-7458.2006.00365.x>
- Holling, C. S. (1959). The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. *The Canadian Entomologist*, 91(5), 293–320. <https://doi.org/10.4039/ent91293-5>
- Hood, G. R., Zhang, L., Hu, E. G., Ott, J. R., & Egan, S. P. (2019). Cascading reproductive isolation: Plant phenology drives temporal isolation among populations of a host-specific herbivore. *Evolution*, 73(3), 554–568. <https://doi.org/10.1111/evo.13683>
- Hudson, R. R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence data. *Genetics*, 132(2), 583–589. <https://doi.org/10.1093/genetics/132.2.583>
- Ingleby, F. C., Hunt, J., & Hosken, D. J. (2010). The role of genotype-by-environment interactions in sexual selection. *Journal of Evolutionary Biology*, 23(10), 2031–2045. <https://doi.org/10.1111/j.1420-9101.2010.02080.x>
- Jacquemyn, H., De Kort, H., Vanden Broeck, A., & Brys, R. (2018). Immigrant and extrinsic hybrid seed inviability contribute to reproductive isolation between forest and dune ecotypes of *Epipactis helleborine* (Orchidaceae). *Oikos*, 127(1), 73–84. <https://doi.org/10.1111/OIK.04329>
- Jauker, F., Diekötter, T., Schwarzbach, F., & Wolters, V. (2009). Pollinator dispersal in an agricultural matrix: Opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. *Landscape Ecology*, 24(4), 547–555. <https://doi.org/10.1007/s10980-009-9331-2>
- Jia, F. Y., & Greenfield, M. D. (1997). When are good genes good? Variable outcomes of female choice in wax moths. *Proceedings of the Royal Society of London B: Biological Sciences*, 264(1384), 1057–1063. <https://doi.org/10.1098/rspb.1997.0146>
- Jin, W. -T., Gernandt, D. S., Wehenkel, C., Xia, X. -X., Wei, X. -X., & Wang, X. -Q. (2021). Phylogenomic and ecological analyses reveal the spatiotemporal evolution of global pines. *Proceedings of the National Academy of Sciences of the United States of America*, 118(20), e2022302118. <https://doi.org/10.1073/pnas.2022302118>
- Kawecki, T. J. (1997). Sympatric speciation via habitat specialization driven by deleterious mutations. *Evolution*, 51(6), 1751–1763. <https://doi.org/10.1111/j.1558-5646.1997.tb05099.x>
- Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, 7(12), 1225–1241. <https://doi.org/10.1111/j.1461-0248.2004.00684.x>
- Kimball, S., Campbell, D. R., & Lessin, C. (2008). Differential performance of reciprocal hybrids in multiple environments. *Journal of Ecology*, 96(6), 1306–1318. <https://doi.org/10.1111/j.1365-2745.2008.01432.x>
- Knerer, G. (1984). Diprionid sawflies: Biological topics and rearing techniques (Hymenoptera: Symphyta). *Bulletin of the Entomological Society of America*, 30(3), 53–57. <https://doi.org/10.1093/besa/30.3.53>
- Knerer, G. (1993). Life history diversity in sawflies. In M. R. Wagner & K. F. Raffa (Eds.), *Sawfly life history adaptations to woody plants* (pp. 33–60). Academic Press.
- Knerer, G., & Atwood, C. E. (1972). Evolutionary trends in the subsoil sawflies belonging to the *Neodiprion abietis* complex (Hymenoptera: Tenthredinoidea). *American Zoologist*, 12(3), 407–418. <https://doi.org/10.2307/3881776>
- Knerer, G., & Atwood, C. E. (1973). Diprionid sawflies: Polymorphism and speciation. *Science*, 179(4078), 1090–1099. <https://doi.org/10.1126/science.179.4078.1090>
- Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, 15(5), 1179–1191. <https://doi.org/10.1111/1755-0998.12387>
- Kühbandner, S., Hacker, K., Niedermayer, S., Steidle, J. L. M., & Ruther, J. (2012). Composition of cuticular lipids in the pteromalid wasp *Lariophagus distinguendus* is host dependent. *Bulletin of Entomological Research*, 102(5), 610–617. <https://doi.org/10.1017/S000748531200017X>
- Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, 82(13), 1–26. <https://doi.org/10.18637/JSS.V082.I13>
- Larsson, S., Björkman, C., & Gref, R. (1986). Responses of *Neodiprion sertifer* (Hym., Diprionidae) larvae to variation in needle resin acid concentration in Scots pine. *Oecologia*, 70(1), 77–84. <https://doi.org/10.1007/BF00377113>
- Lemic, D., Mikac, K. M., Ivkovic, S. A., & Bažok, R. (2015). The temporal and spatial invasion genetics of the Western corn rootworm (Coleoptera: Chrysomelidae) in Southern Europe. *PLoS One*, 10(9), e0138796. <https://doi.org/10.1371/journal.pone.0138796>
- Lenth, R. V. (2020). *emmeans: Estimated marginal means, aka least-squares means*. <https://CRAN.R-project.org/package=emmeans>
- Levin, D. A. (2009). Flowering-time plasticity facilitates niche shifts in adjacent populations. *New Phytologist*, 183(3), 661–666. <https://doi.org/10.1111/j.1469-8137.2009.02889.x>
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R.; 1000 Genome Project Data Processing Subgroup. (2009). The sequence Alignment/Map format and SAMtools. *Bioinformatics*, 25(16), 2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
- Liao, Y. C., Huang, S. S., & Yang, M. M. (2016). Substrate-borne signals, specific recognition, and plant effects on the acoustics of two allied species of *Trioza*, with the description of a new species (Psylloidea: Triozidae). *Annals of the Entomological Society of America*, 109(6), 906–917. <https://doi.org/10.1093/aesa/saw060>
- Lindstedt, C., Bagley, R. K., Calhim, S., Jones, M., & Linnen, C. R. (2022). The impact of life stage and pigment source on the evolution of novel warning signal traits. *Evolution*, 76(3), 554–572. <https://doi.org/10.1111/evo.14443>
- Linn, C. E., Dambroski, H. R., Feder, J. L., Berlocher, S. H., Nojima, S., & Roelofs, W. L. (2004). Postzygotic isolating factor in sympatric speciation in *Rhagoletis* flies: Reduced response of hybrids to parental host-fruit odors. *Proceedings of the National Academy of Sciences of the United States of America*, 101(51), 17753–17758. <https://doi.org/10.1073/pnas.0408255101>
- Linnen, C. R., & Farrell, B. D. (2010). A test of the sympatric host race formation hypothesis in *Neodiprion* (Hymenoptera: Diprionidae). *Proceedings of the Royal Society B: Biological Sciences*, 277(1697), 3131–3138. <https://doi.org/10.1098/rspb.2010.0577>
- Linnen, C. R., O'Quinn, C. T., Shackleford, T., Sears, C. R., & Lindstedt, C. (2018). Genetic basis of body color and spotting pattern in red-headed pine sawfly larvae (*Neodiprion lecontei*). *Genetics*, 209(1), 291–305. <https://doi.org/10.1534/genetics.118.300793>

- Mancilla-Morales, M. D., Velarde, E., Aguilar, A., Contreras-Rodríguez, A., Ezcurra, E., Rosas-Rodríguez, J. A., Soñanez-Organis, J. G., & Ruiz, E. A. (2022). Strong philopatry, isolation by distance, and local habitat have promoted genetic structure in Heermann's Gull. *Diversity*, 14(2), 108. <https://doi.org/10.3390/d14020108>
- Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, 27(2), 209–220.
- Martin, S. H., Dasmahapatra, K. K., Nadeau, N. J., Salazar, C., Walters, J. R., Simpson, F., Blaxter, M., Manica, A., Mallet, J., & Jiggins, C. D. (2013). Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Research*, 23(11), 1817–1828. <https://doi.org/10.1101/gr.159426.113>
- Matsubayashi, K. W., Oshima, I., & Nosil, P. (2010). Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata*, 134(1), 1–27. <https://doi.org/10.1111/j.1570-7458.2009.00916.x>
- Matthysen, E. (2013). Multicausality of dispersal: A review. In J. Clobert, M. Baguette, T. G. Benton, & J. M. Bullock (Eds.), *Dispersal ecology and evolution* (pp. 3–18). Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199608898.003.0001>
- McBride, C. S., & Singer, M. C. (2010). Field studies reveal strong postmating isolation between ecologically divergent butterfly populations. *PLoS Biology*, 8(10), e1000529. <https://doi.org/10.1371/journal.pbio.1000529>
- Meirmans, P. G. (2012). The trouble with isolation by distance. *Molecular Ecology*, 21(12), 2839–2846. <https://doi.org/10.1111/j.1365-294X.2012.05578.x>
- Merrick, M. J., & Koprowski, J. L. (2016). Evidence of natal habitat preference induction within one habitat type. *Proceedings of the Royal Society B: Biological Sciences*, 283(1842), 20162106. <https://doi.org/10.1098/rspb.2016.2106>
- Minchin, P. R. (1987). An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio*, 69(1–3), 89–107. <https://doi.org/10.1007/bf00038690>
- Moncada, B., Mercado-Díaz, J. A., Magain, N., Hodkinson, B. P., Smith, C. W., Bungartz, F., Pérez-Pérez, R. E., Gumboski, E., Sérusiaux, E., Lumbsch, H. T., & Lücking, R. (2021). Phylogenetic diversity of two geographically overlapping lichens: Isolation by distance, environment, or fragmentation? *Journal of Biogeography*, 48(3), 676–689. <https://doi.org/10.1111/JBI.14033>
- Moore, R. P., Robinson, W. D., Lovette, I. J., & Robinson, T. R. (2008). Experimental evidence for extreme dispersal limitation in tropical forest birds. *Ecology Letters*, 11(9), 960–968. <https://doi.org/10.1111/j.1461-0248.2008.01196.x>
- Morales, A. E., Jackson, N. D., Dewey, T. A., O'Meara, B. C., & Carstens, B. C. (2017). Speciation with gene flow in North American *Myotis* bats. *Systematic Biology*, 66(3), 440–452. <https://doi.org/10.1093/sysbio/syw100>
- Moreira, X., Mooney, K. A., Rasmann, S., Petry, W. K., Carrillo-Gavilán, A., Zas, R., & Sampedro, L. (2014). Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecology Letters*, 17(5), 537–546. <https://doi.org/10.1111/ele.12253>
- Moreira, X., Zas, R., & Sampedro, L. (2012). Differential allocation of constitutive and induced chemical defenses in pine tree juveniles: A test of the optimal defense theory. *PLoS One*, 7(3), e34006. <https://doi.org/10.1371/journal.pone.0034006>
- Nerg, A., Kainulainen, P., Vuorinen, M., Hanso, M., Holopainen, J. K., & Kärkelä, T. (1994). Seasonal and geographical variation of terpenes, resin acids and total phenolics in nursery grown seedlings of Scots pine (*Pinus sylvestris* L.). *New Phytologist*, 128(4), 703–713. <https://doi.org/10.1111/j.1469-8137.1994.tb04034.x>
- Nosil, P. (2008). Speciation with gene flow could be common. *Molecular Ecology*, 17(9), 2103–2106. <https://doi.org/10.1111/j.1365-294X.2008.03715.x>
- Nosil, P. (2012). *Ecological speciation*. Oxford University Press.
- Nosil, P., Vines, T. H., & Funk, D. J. (2005). Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, 59(4), 705–719. <https://doi.org/10.1554/04-428>
- Nylin, S., & Gotthard, K. (2003). Plasticity in life-history traits. *Annual Review of Entomology*, 43(1), 63–83. <https://doi.org/10.1146/annurev.ento.43.1.63>
- Oksanen, J. (2010). *Vegan: Community ecology package*. <https://CRAN.R-project.org/package=vegan>
- Olofsson, E. (1987). Mortality factors in a population of *Neodiprion sertifer* (Hymenoptera: Diprionidae). *Oikos*, 48(3), 297. <https://doi.org/10.2307/3565517>
- Papadopoulos, A. S. T., Baker, W. J., Crayn, D., Butlin, R. K., Kynast, R. G., Hutton, I., & Savolainen, V. (2011). Speciation with gene flow on Lord Howe Island. *Proceedings of the National Academy of Sciences of the United States of America*, 108(32), 13188–13193. <https://doi.org/10.1073/pnas.1106085108>
- Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, 7(5), e37135. <https://doi.org/10.1371/journal.pone.0037135>
- Peterson, M. A., & Denno, R. F. (1998). The influence of dispersal and diet breadth on patterns of genetic isolation by distance in phytophagous insects. *The American Naturalist*, 152(3), 428–446. <https://doi.org/10.1086/286180>
- Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution*, 25(8), 459–467. <https://doi.org/10.1016/j.tree.2010.05.006>
- Pflüger, F. J., & Balkenhol, N. (2014). A plea for simultaneously considering matrix quality and local environmental conditions when analysing landscape impacts on effective dispersal. *Molecular Ecology*, 23(9), 2146–2156. <https://doi.org/10.1111/mec.12712>
- Pinho, C., & Hey, J. (2010). Divergence with gene flow: Models and data. *Annual Review of Ecology, Evolution, and Systematics*, 41(1), 215–230. <https://doi.org/10.1146/annurev-ecolsys-102209-144644>
- Pires, T. H. S., Borghezan, E. A., Cunha, S. L. R., Leitão, R. P., Pinto, K. S., & Zuanon, J. (2019). Sensory drive in colourful waters: Morphological variation suggests combined natural and sexual selection in an Amazonian fish. *Biological Journal of the Linnean Society*, 127(2), 351–360. <https://doi.org/10.1093/biolinnean/blz054>. <https://academic.oup.com/biolinnean/article/127/2/351/5480936>
- Potkamp, G., & Franssen, C. H. (2019). Speciation with gene flow in marine systems. *Contributions to Zoology*, 88(2), 1–40. <https://doi.org/10.1163/18759866-20191344>
- Powell, T. H. Q., Forbes, A. A., Hood, G. R., & Feder, J. L. (2014). Ecological adaptation and reproductive isolation in sympatry: Genetic and phenotypic evidence for native host races of *Rhagoletis pomonella*. *Molecular Ecology*, 23(3), 688–704. <https://doi.org/10.1111/mec.12635>
- Prunier, R., Akman, M., Kremer, C. T., Aitken, N., Chuah, A., Borevitz, J., & Holsinger, K. E. (2017). Isolation by distance and isolation by environment contribute to population differentiation in *Protea repens* (Proteaceae L.), a widespread South African species. *American Journal of Botany*, 104(5), 674–684. <https://doi.org/10.3732/ajb.1600232>
- R Core Team. (2022). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. <http://www.r-project.org/>
- Rafferty, N. E., Diez, J. M., & Bertelsen, C. D. (2020). Changing climate drives divergent and nonlinear shifts in flowering phenology across elevations. *Current Biology*, 30(3), 432–441.e3. <https://doi.org/10.1016/j.cub.2019.11.071>
- Ronce, O., & Clobert, J. (2013). Dispersal syndromes. In J. Clobert, M. Baguette, T. G. Benton, & J. M. Bullock (Eds.), *Dispersal ecology and evolution* (pp. 119–138). Oxford University Press. <https://doi.org/10.1093/acprof:oso/9780199608898.003.0010>
- Rundle, H. D., Chenoweth, S. F., Doughty, P., & Blows, M. W. (2005). Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology*, 3(11), e368. <https://doi.org/10.1371/journal.pbio.0030368>

- Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, 8(3), 336–352. <https://doi.org/10.1111/j.1461-0248.2004.00715.x>
- Sandoval, C. P. (1994a). Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae: Timemidae) and its consequences for host range. *Biological Journal of the Linnean Society*, 52(4), 341–356. <https://doi.org/10.1111/j.1095-8312.1994.tb00996.x>
- Sandoval, C. P. (1994b). The effects of the relative geographic scales of gene flow and selection on morph frequencies in the walking stick *Timema cristinae*. *Evolution*, 48(6), 1866–1879. <https://doi.org/10.1111/j.1558-5646.1994.tb02220.x>
- Santos, H., Burban, C., Rousselet, J., Rossi, J. P., Branco, M., & Kerdellu , C. (2011). Incipient allochronic speciation in the pine processionary moth (*Thaumetopoea pityocampa*, Lepidoptera, Notodontidae). *Journal of Evolutionary Biology*, 24(1), 146–158. <https://doi.org/10.1111/j.1420-9101.2010.02147.x>
- Sattman, D. A., & Cocroft, R. B. (2003). Phenotypic plasticity and repeatability in the mating signals of *Enchenopa* treehoppers, with implications for reduced gene flow among host-shifted populations. *Ethology*, 109(12), 981–994. <https://doi.org/10.1046/j.1439-0310.2003.00940.x>
- Schmidt, T. L., Swan, T., Chung, J., Karl, S., Demok, S., Yang, Q., Field, M. A., Muzari, M. O., Ehlers, G., Brugh, M., Bellwood, R., Horne, P., Burkot, T. R., Ritchie, S., & Hoffmann, A. A. (2021). Spatial population genomics of a recent mosquito invasion. *Molecular Ecology*, 30(5), 1174–1189. <https://doi.org/10.1111/mec.15792>
- Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 Years of image analysis. *Nature Methods*, 9(7), 671–675. <https://doi.org/10.1038/nmeth.2089>
- Schweyen, H., Rozenberg, A., & Leese, F. (2014). Detection and removal of PCR duplicates in population genomic ddRAD studies by addition of a degenerate base region (DBR) in sequencing adapters. *Biological Bulletin*, 227(2), 146–160. <https://doi.org/10.1086/BBLv227n2p146>
- Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., Van Der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H., & Okada, N. (2008). Speciation through sensory drive in cichlid fish. *Nature*, 455(7213), 620–626. <https://doi.org/10.1038/nature07285>
- Sekhon, J. (2008). Multivariate and propensity score matching software with automated balance optimization: The Matching package for R. *Journal of Statistical Software*, 42(7), 1–52. <https://doi.org/10.18637/jss.v042.i07>
- Servedio, M. R. (2004). The evolution of premating isolation: Local adaptation and sexual selection against hybrids. *Evolution*, 58(5), 913–924. <https://doi.org/10.1111/j.0014-3820.2004.tb00425.x>
- Servedio, M. R., & Noor, M. A. F. (2003). The role of reinforcement in speciation: Theory and data. *Annual Review of Ecology, Evolution, and Systematics*, 34, 339–364. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132412>
- Sexton, J. P., Hangartner, S. B., & Hoffmann, A. (2014). Genetic isolation by environment or distance: Which pattern of gene flow is most common? *Evolution*, 68(1), 1–15. <https://doi.org/10.1111/evo.12258>
- Shafer, A. B. A., & Wolf, J. B. W. (2013). Widespread evidence for incipient ecological speciation: A meta-analysis of isolation-by-ecology. *Ecology Letters*, 16(7), 940–950. <https://doi.org/10.1111/ele.12120>
- Silvertown, J., Servaes, C., Biss, P., & Macleod, D. (2005). Reinforcement of reproductive isolation between adjacent populations in the Park Grass Experiment. *Heredity*, 95(3), 198–205. <https://doi.org/10.1038/sj.hdy.6800710>
- Singer, M. C., Thomas, C. D., & Parmesan, C. (1993). Rapid human-induced evolution of insect–host associations. *Nature*, 366(6456), 681–683. <https://doi.org/10.1038/366681a0>
- Smadja, C. M., & Butlin, R. K. (2011). A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology*, 20(24), 5123–5140. <https://doi.org/10.1111/j.1365-294X.2011.05350.x>
- Smith, D. R. (1993). Systematics, life history, and distribution of sawflies. In M. R. Wagner & K. F. Raffa (Eds.), *Sawfly life history adaptations to woody plants* (pp. 3–32). Academic Press.
- Smouse, P. E., Long, J. C., & Sokal, R. R. (1986). Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology*, 35(4), 627–632. <https://doi.org/10.2307/2413122>
- Sokal, R. R. (1979). Testing statistical significance of geographic variation patterns. *Systematic Zoology*, 28(2), 227–232. <https://doi.org/10.2307/2412528>
- Sousa, V. C., Z  l  , F., Rodrigues, L. R., Godinho, D. P., Charlerly de la Masseli  re, M., & Magalh  es, S. (2019). Rapid host-plant adaptation in the herbivorous spider mite *Tetranychus urticae* occurs at low cost. *Current Opinion in Insect Science*, 36, 82–89. <https://doi.org/10.1016/j.cois.2019.08.006>
- Svenning, J. -C., Normand, S., & Skov, F. (2008). Postglacial dispersal limitation of widespread forest plant species in nemoral Europe. *Ecography*, 31(3), 316–326. <https://doi.org/10.1111/j.0906-7590.2008.05206.x>
- Svensson, G. P., Althoff, D. M., & Pellmyr, O. (2005). Replicated host-plant formation in bogus yucca moths: Genetic and ecological divergence of *Prodoxus quinquepunctellus* on yucca hosts. *Evolutionary Ecology Research*, 7(8), 1139–1151.
- Sydenham, M. A. K., Moe, S. R., Kuhlmann, M., Potts, S. G., Roberts, S. P. M., Totland, O., & Eldegard, K. (2017). Disentangling the contributions of dispersal limitation, ecological drift, and ecological filtering to wild bee community assembly. *Ecosphere*, 8(1), e01650. <https://doi.org/10.1002/ecs2.1650>
- Taylor, R. S., & Friesen, V. L. (2017). The role of allochrony in speciation. *Molecular Ecology*, 26(13), 3330–3342. <https://doi.org/10.1111/mec.14126>
- Thibert-Plante, X., & Hendry, A. P. (2011). Factors influencing progress towards sympatric speciation. *Journal of Evolutionary Biology*, 24(10), 2186–2196. <https://doi.org/10.1111/j.1420-9101.2011.02348.x>
- Thomas, Y., Bethenod, M. T., Pelozuelo, L., Fr  rot, B., & Bourguet, D. (2003). Genetic isolation between two sympatric host-plant races of the European corn borer, *Ostrinia nubilalis* Hubner I. Sex pheromone, moth emergence timing, and parasitism. *Evolution*, 57(2), 261–273. <https://doi.org/10.1111/j.0014-3820.2003.tb00261.x>
- Thompson, J. N. (1998). Rapid evolution as an ecological process. *Trends in Ecology & Evolution*, 13(8), 329–332. [https://doi.org/10.1016/s0169-5347\(98\)01378-0](https://doi.org/10.1016/s0169-5347(98)01378-0)
- Thompson, K. A., Urquhart-Cronish, M., Whitney, K. D., Rieseberg, L. H., & Schluter, D. (2021). Patterns, predictors, and consequences of dominance in hybrids. *The American Naturalist*, 197(3), E72–E88. <https://doi.org/10.1086/712603>
- Tisdale, R. A., & Wagner, M. R. (1991). Oviposition behavior of *Neodiprion fulviceps* (Cresson) (Hymenoptera: Diprionidae) on ponderosa pine. *Journal of Insect Behavior*, 4(5), 609–617. <https://doi.org/10.1007/bf01048073>
- Van Wagner, C. E. (1967). *Seasonal variation in moisture content of eastern Canadian tree foliage and the possible effect on crown fires* (Forestry Branch Departmental Publication 1204, p. 22). Government of Canada Department of Forestry and Rural Development.
- Vertacnik, K. L., Geib, S. M., & Linnen, C. R. (2016). *Neodiprion lecontei* genome assembly Nlec1.0. (dataset). NCBI/GenBank. <http://dx.doi.org/10.15482/USDA.ADC/1235565>
- Via, S. (2001). Sympatric speciation in animals: The ugly duckling grows up. *Trends in Ecology and Evolution*, 16(7), 381–390. [https://doi.org/10.1016/s0169-5347\(01\)02188-7](https://doi.org/10.1016/s0169-5347(01)02188-7)
- Via, S., Bouck, A. C., & Skillman, S. (2000). Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution*, 54(5), 1626–1637. <https://doi.org/10.1111/j.0014-3820.2000.tb00707.x>
- Wang, I. J. (2013). Examining the full effects of landscape heterogeneity on spatial genetic variation: A multiple matrix regression approach for quantifying geographic and ecological isolation. *Evolution*, 67(12), 3403–3411. <https://doi.org/10.1111/evo.12134>
- Wang, I. J., & Bradburd, G. S. (2014). Isolation by environment. *Molecular Ecology*, 23(23), 5649–5662. <https://doi.org/10.1111/mec.12938>

- Wang, I. J., Glor, R. E., & Losos, J. B. (2013). Quantifying the roles of ecology and geography in spatial genetic divergence. *Ecology Letters*, 16(2), 175–182. <https://doi.org/10.1111/ele.12025>
- Wang, I. J., & Summers, K. (2010). Genetic structure is correlated with phenotypic divergence rather than geographic isolation in the highly polymorphic strawberry poison-dart frog. *Molecular Ecology*, 19(3), 447–458. <https://doi.org/10.1111/j.1365-294X.2009.04465.x>
- Weber, J. N., Bradburd, G. S., Stuart, Y. E., Stutz, W. E., & Bolnick, D. I. (2017). Partitioning the effects of isolation by distance, environment, and physical barriers on genomic divergence between parapatric threespine stickleback. *Evolution*, 71(2), 342–356. <https://doi.org/10.1111/evo.13110>
- Wilson, L. F., Wilkinson, R. C., & Averill, R. C. (1992). *Redheaded pine sawfly—Its ecology and management* (Agricultural Handbook 694, p. 54). United States Department of Agriculture.
- Wood, T. K., Tilmon, K. J., Shantz, A. B., Harris, C. K., & Pesek, J. (1999). The role of host-plant fidelity in initiating insect race formation. *Evolutionary Ecology Research*, 1(3), 317–322.
- Wright, S. (1943). Isolation by distance. *Genetics*, 28(2), 114–138. <https://doi.org/10.1093/genetics/28.2.114>
- Wright, S. (1946). Isolation by distance under diverse systems of mating. *Genetics*, 31(1), 39–59. <https://doi.org/10.1093/genetics/31.1.39>
- Zhang, L., Driscoe, A., Izen, R., Toussaint, C., Ott, J. R., & Egan, S. P. (2017). Immigrant inviability promotes reproductive isolation among host-associated populations of the gall wasp *Belonocnema treatae*. *Entomologia Experimentalis et Applicata*, 162(3), 379–388. <https://doi.org/10.1111/eea.12548>
- Zhang, Y. M., Bass, A. I. H., Fernández, D. C., & Sharanowski, B. J. (2018). Habitat or temporal isolation: Unraveling herbivore–parasitoid speciation patterns using double digest RADseq. *Ecology and Evolution*, 8(19), 9803–9816. <https://doi.org/10.1002/ece3.4457>