Sequential Sympatric Speciation Across Trophic Levels

Andrew A. Forbes,1,4 Thomas H.Q. Powell,3 Lukasz L. Stelinski,2 James J. Smith,3 Jeffrey L. Feder1,∗†

A major cause for biodiversity may be biodiversity itself. As new species form, they may create new niches for others to exploit, potentially catalyzing a chain reaction of speciation events across trophic levels. We tested for such sequential radiation in the Rhoagletis pomonella (Diptera: Tephritidae) complex, a model for sympatric speciation via host shifting. We report that the parasitic wasp Diachasma alloeum (Hymenoptera: Braconidae) has formed new incipient species as a result of specializing on diversifying fly hosts, including the recently derived apple-infesting race of R. pomonella. Furthermore, we show that traits that differentially adapt R. pomonella flies to their host plants also have quickly evolved and serve as ecological barriers to reproduction, isolating the wasps. Speciation therefore cascades as the effects of new niche construction move across trophic levels.

The idea that species induce speciation has been inferred to explain current and past patterns of biodiversity by paleontologists, ecologists, and evolutionary biologists alike (1–3). However, this hypothesis of sequential radiation is difficult to directly test in nature. Examples such as adaptive radiations after mass extinctions (4), species richness in the tropics (5), and the increased diversity of insects having herbivorous life styles (5, 6) have mainly been investigated on the basis of phylogenetic inference and/or correlative analyses.

Host plant–specific phytophagous insects and their parasites may be good candidates for testing the sequential radiation hypothesis (7). This is because new resource opportunities become available when a plant-eating insect diversifies by shifting and adapting to a novel host plant, with its guild of associated parasites potentially following suit and speciating in kind. Unfortunately, a lack of historical and biogeographic information concerning host shifting and the absence of a free-living parasite life stage often complicate our understanding of plant-insect-parasite systems. In these cases, cocladogenesis (cospeciation resulting from parallel allopatry of interacting organisms) rather than the cascading effects of shifting host ecology across trophic levels could trigger codiversification(8). One cannot rule out that insect and parasitoids became separated in tandem from other conspecifics and evolved into new species as a consequence of their shared physical isolation.

References and Notes

29. Y. Iguchi, Kogane 1, 21 (2000).
36. We thank S. Spector, T. Larsen, A. Solis, T. Gardner, J. Louzada, P. Skelley, D. Almqist, C. Gillett, D. Edmonds, D. Lewis, L. Herman, T. Fincher, M. Barclay, C. O’Brien, J. Prena, F. Génier, G. J. and J. Llewellyn, R. Veal, D. Heinicke, and B. Raber for providing critical samples; assistance of the Scarabaeine Research Network; K. Bright, W. Eberhard, E. Greene, A. Kodric-Brown, T. Maginnis, E. McCullough, C. Qualls, and two anonymous reviewers for ideas and comments on the manuscript; J. Driver and the University of Montana Electron Microscopy facility for the weevil images; and the NSF (IOS-0564240) (to D.J.E.) for funding.

Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5915/773/DC1

Materials and Methods

Figs. 1 and 2

17 October 2008; accepted 7 January 2009
10.1126/science.1167345

†Department of Biological Sciences, University of Notre Dame, Galvin Life Sciences Building, Notre Dame, IN 46556, USA.
‡Department of Entomology and Nematology, University of Florida Citrus Research and Education Center, 700 Experiment Station Road, Lake Alfred, FL 33850, USA.
§Department of Entomology and Lyman Briggs College, Michigan State University, East Lansing, MI 48824, USA.
*Present address: Department of Entomology, University of California at Davis, One Shields Avenue, Davis, CA 95616, USA. To whom correspondence should be addressed. E-mail: aforbes@ucdavis.edu
**Present address: Wissenschaftskolleg zu Berlin Institute for Advanced Study, Wallotstrasse 19, D14193 Berlin, Germany.
The *Rhagoletis pomonella* sibling species complex is a model for speciation in the absence of geographic isolation via host plant shifting. The natural history of these flies, including the recent sympatric host shift of the species *R. pomonella* from hawthorn to introduced, domesticated apple within the past 150 years (9), suggests that they did not evolve as a result of passive cocladogenesis. Therefore, we tested for sequential radiation of its specialist parasitoid wasp, *Diaschisma alloeum* [see supporting online material (SOM) text for parallels between fly and wasp biology], by examining whether wasps attacking the ancestral hawthorn and the derived apple races *R. pomonella*, as well as the closely related sibling species *R. mendax* (host: blueberry, *Vaccinium* spp.) and *R. zephyria* (snowberry, *Symphoricarpos* spp.), display patterns of host-related genetic variation similar to those of the flies. *D. alloeum* only attacks *R. pomonella* complex flies found on these four host plants (10). We also investigated whether wasps differed by the same type of host–plant–specific mating and diapause traits that are ecologically isolating the flies.

The pattern of genetic differentiation among *D. alloeum* populations was similar to that for *R. pomonella* flies (11, 12). Mitochondrial DNA (mtDNA) cytochrome oxidase I (COI) sequences displayed only modest host-related differentiation for the wasps (Fig. 1A, figs. S1 and S2, and tables S1 and S2). Therefore, the wasps are apparently of relatively recent origin and do not represent highly genetically diverged cryptic sibling species. However, we did find a mtDNA haplotype in snowberry wasps that was not found in the other wasp populations (fig. S2 and table S2). In addition, a common mtDNA haplotype in apple, hawthorn, and blueberry wasps was not present in the snowberry population. Thus, in a mtDNA genetic distance network, the snowberry wasp was offset from the other taxa (Fig. 1A).

Nine of 21 microsatellite loci analyzed (DA003, DA013, DA019, DA150, DA174, DA183, DA192, DA202, and DA205) displayed consistent host-related allele frequency differences between at least two of the wasp populations (table S3 and SOM appendix), much like allozymes for *R. pomonella* flies (13, 14). Locus DA003 showed particularly pronounced frequency differences (see Fig. 2 for graph of combined DA003 196+/200+204 allele frequencies). Indeed, allele 196 was common in apple (mean frequency = 0.292; *n* = 291 total alleles scored), blueberry (0.179, *n* = 246), and snowberry (0.257, *n* = 101) wasp populations but absent from all hawthorn wasps (*n* = 385 alleles scored; SOM appendix). The lack of allele 196 in hawthorn wasps for locus DA003 suggests that there is little or no effective interhost gene flow from apple, blueberry, or snowberry wasp populations into the hawthorn population. Neighbor-joining trees for the nine loci displaying host-related divergence (Fig. 1B) and the full 21-loci data set (fig. S3) separated hawthorn and blueberry wasp populations at different ends of the networks. The lone snowberry wasp site analyzed was genetically most closely associated with blueberry wasps, whereas the apple wasps were placed at an intermediate position between blueberry and hawthorn populations (Fig. 1B).

We next investigated whether the same host-related adaptations ecologically isolating *R. pomonella* flies were responsible for genetically differentiating the wasps. Because *Rhagoletis* flies mate on or near the fruit of their respective host plants (15–17), host choice generates prezygotic isolation and facilitates the evolution of host-specific performance traits. A field study of free-living adult *D. alloeum* in a blueberry patch in Fennville, MI, suggested that the wasps also use host fruit as a rendezvous site for mating. Before copulation, all male and female wasps that formed the 24 mating pairs recorded in the study were first observed flying toward blueberries from distances of 1 to 3 m away. In all cases, wasps flew close to blueberries, sometimes making contact with the fruit, before they initiated coupling on nearby host leaves (mean distance mating from fruit = 7.4 ± 6.3 cm (SD); *n* = 24, range = 3 to 30 cm). The average time from first observation to mating was 172.2 ± 94.7 s (SD). *Rhagoletis* flies use the volatile compounds emitted from the surface of ripening fruit as olfactory cues to both find and discriminate among host plants for mating and oviposition (18). We thus tested whether wasps show similar discriminatory behavior for surface host fruit volatiles by using a y-tube olfactometer (fig. S4). We found that naive adult wasps from apple, hawthorn, and blueberry populations positively oriented to the arm of the y-tube containing their natal fruit odor and were antagonized by nonnatal volatiles (Table 1). There was no qualitative difference between males and females in their behavioral responses to surface fruit volatiles. However, female hawthorn wasps displayed stronger preferences for natal hawthorn fruit volatiles (Fisher’s exact test, *P* = 0.006, 1 df) and more pronounced antagonism to nonnatal apple volatiles (*P* = 0.030, 1 df) than male hawthorn wasps did. Snowberry wasps showed behavioral specificity for their natal fruit volatiles that was similar to that of the other parasites, but too few snowberry wasps were available for testing to draw definitive conclusions (Table 1). All wasps exhibited behavioral antagonism to the volatiles of flowering dogwood fruit (*Cornus florida*), a host for an undescribed sister taxon to *R. pomonella* that *D. alloeum* does not attack (19). These data suggest that fruit odor discrimination may act in a similar manner in the wasps as in the flies and may generate host-specific mating, resulting in an ecological barrier to gene flow in sympatry. Because we cannot mate or rear *D. alloeum* for multiple generations in the laboratory, it is unknown whether maternal effects and/or larval conditioning may contribute to genetically based fruit volatile discrimination. In the rare instances in which larval experience was inferred to influence an adult insect’s preference, the effect was mediated via direct larval contact with plant compounds (20). But, because *D. alloeum* has no direct contact with surface fruit volatiles at any stage of its life before adult eclosion, larval conditioning is unlikely.

Diapause life-history differences represent a second critical ecological barrier to gene flow among *Rhagoletis* flies (21). Sympatric blueberry, apple, and hawthorn flies all eclose as adults at different times in the spring and/or summer, matching the timing of fruit ripeness on their respective

**Fig. 1.** Genetic distance networks depicting relationships of *D. alloeum* wasp populations attacking blueberry (blue), apple (green), hawthorn (red), and snowberry flies (black). (A) Fitch network created on the basis of Nei genetic distances of mtDNA haplotype frequencies in wasp populations. (B) Neighbor-joining Nei genetic distance network of the nine microsatellite loci showing consistent site-to-site host-related frequency differences. Black ovals give bootstrap confidence values (10,000 replicates). Site abbreviations are MGR = Grant, MI; MHL = Holland, MI; MEL = East Lansing, MI; MSI = Fennville, MI; MDW = Dowagiac, MI; IL = Urbana, IL; PASC = State College, PA; NJCW = Chatsworth, NJ. See fig. S1 and table S1 for a map and descriptions, respectively, of study sites.
host plants (Fig. 3). Because *Rhagoletis* flies have only one generation per year and live for about a month in the field, the differences in the timing of eclosion can allochronically isolate the flies. Laboratory rearing experiments and field studies of *D. alloeum* indicated that similar eclosion time differences exist among blueberry, apple, and hawthorn wasps (Fig. 3 and table S4). The lone exception was a late fruiting apple from the Dowagiac, MI site where apple wasps had a relatively late mean eclosion time of 112.9 ± 1.01 days (SE) (n = 102) that overlapped with that of hawthorn wasps (111.1 ± 1.13 days, n = 44). Mean longevity of adult *D. alloeum* was determined to be less than 2 weeks in the laboratory (12.9 ± 0.4 days SE, n = 286). Assuming wasps have a similar lifespan in nature (a likely overestimate), eclosion time differences may decrease seasonal overlap and mating opportunities between blueberry and apple wasps at the sympatric Fennville, MI site by 28.9%, between apple and hawthorn wasps by 29.8%, and between blueberry and hawthorn wasps by 74.5%.

For *R. pomonella*, the allozymes displaying host-related frequency differences correlate with eclosion time (9), tying together the genetics of host race formation with a trait that allochronically isolates the flies. We tested for similar relationships for the microsatellite loci separately in male and female wasps because of sex-related differences in eclosion time (male wasps eclose several days earlier than females). For *D. alloeum*, the microsatellite loci were significantly correlated with variation in eclosion time for hawthorn (r² stepwise multiple regression for males = 0.504, P < 0.0001, 52 df; r² males = 0.525, P < 0.0001, 46 df), apple (r² females = 0.163, P = 0.0097, 54 df; r² males = 0.657, P < 0.0001, 30 df), and blueberry (r² females = 0.686, P < 0.0001, 29 df; r² males = 0.511, P < 0.0001, 53 df; table S5) wasps. In particular, the locus DA003 was significantly correlated with eclosion time variation in blueberry wasps (r² females = 0.378, P = 0.0003, 29 df; r² males = 0.511, P < 0.0001, 53 df) but only moderately in female apple wasps (r² females = 0.101, P = 0.0182, 54 df) and was not a significant predictor for hawthorn wasps. The alleles 16F+200+204 at locus DA003 were associated with earlier eclosion times in both female and male blueberry and apple wasps (fig. S5). These three alleles were present in highest frequency for blueberry wasps (0.790, n = 205 total alleles scored), intermediate for apple (0.485, n = 260), and lowest for hawthorn wasps (0.029, n = 385), corresponding to the order of eclosion from earliest to latest for these populations (Fig. 3). Locus DA003 therefore may represent a naturally segregating, major effect quantitative trait locus for diapause that is associated with ecological reproductive isolation among *D. alloeum* populations.

Our results imply that sympatric host shifts of *R. pomonella* onto new plants may have triggered a reciprocal and rapid starburst of adaptive radiation for its parasitoid, *D. alloeum*. We found that host-related ecological effects initiating speciation for *Rhagoletis* rippled through the community and may have amplified diversity for the wasp. Although we were unable to unambiguously resolve the source of the recently formed apple wasp race in this study, the absence of the 196 allele at microsatellite locus DA003 in the hawthorn wasp race suggests that the hawthorn wasp race is not the sole progenitor of the apple wasps. Together with the mtDNA data, allele 196 and its association with early eclosion instead suggest that blueberry wasps may have given rise to the apple wasp population. It is possible, however, that apple wasps are hybrids resulting from crosses between hawthorn and blueberry wasps on the basis of both their intermediate position in the microsatellite distance network and their extreme overlapping eclosion time. Regardless, our study demonstrates that the origin of the apple wasp was not a result of strict 1:1 coalesadogenesis with apple-infesting *Rhagoletis* flies. One intriguing possibility is that the wasps themselves may be promoting *Rhagoletis* host shifts. Lower parasitism rates for *R. pomonella* in derived hosts like apple [i.e., enemy-free space (22)] could favor race formation for the fly, creating new niches promoting wasp divergence. We must also determine whether non-host-related premating and intrinsic postzygotic isolation exist in the genus *Diachasma*. The sequential radiation hypothesis predicts that if nonecological barriers exist they should arise after host-related barriers evolve.

We have presented one case study supporting sequential speciation. Other studies have identified potential examples (7), and, because *D. alloeum* is just one member of a guild of parasitic braconids attacking *R. pomonella*, it is possible that sympatric host races of *Diachasmimorpha mellea*
Evolution of the Drosophila Nuclear Pore Complex Results in Multiple Hybrid Incompatibilities

Shanwu Tang1 and Daven C. Presgraves1,2

Speciation often involves the evolution of incompitable gene interactions that cause sterility or lethality in hybrids between populations. These so-called hybrid incompatibilities occur between two or more functionally divergent loci. We show that the nucleoporin 160kDa (Nup160) gene of the fruitfly Drosophila simulans is incompatible with one or more factors on the D. melanogaster X chromosome, causing hybrid lethality. Nup160 encodes a nuclear pore complex protein and shows evidence of adaptive evolution. Furthermore, the protein encoded by Nup160 directly interacts with that of another hybrid lethality gene, Nup96, indicating that at least two lethal hybrid incompatibility genes have evolved by byproducts of divergent coevolution among interacting components of the Drosophila nuclear pore complex.

The evolution and genetics of these hybrid incompatibilities have been shown to follow specific rules. For instance, hybrid incompatibilities tend to accumulate gradually as species diverge (3); behave as partial recessives in hybrids (4); follow Haldane’s rule (that is, the preferential sterility or inviability of hybrids of the heterogametic (XY or ZW) sex (5)); and accumulate disproportionately on the X chromosome (that is, the large X-effect) (6, 7). The molecular biology of hybrid incompatibilities has revealed that five of six hybrid incompatibility genes identified so far show signatures of recurrent adaptive evolution (8–13), which suggests that hybrid sterility and inviability generally evolve as incidental byproducts of positive natural selection.

To test whether this emerging molecular rule of speciation holds for additional hybrid incompatibility loci, we performed a genetic screen for lethal hybrid incompatibilities between Drosophila melanogaster and D. simulans, two species that diverged ~3 million years ago. Because all hybrids between D. melanogaster and...