

Phylogeography and Host Association in a Pollinating Seed Parasite, *Greya politella***Jonathan M. Brown, John N. Thompson, Olle Pellmyr, and Richard G. Harrison**

Abstract. We performed a phylogeographic analysis of mitochondrial DNA haplotypes from the pollinating seed parasite *Greya politella* (Lepidoptera: Prodoxidae) in order to determine the degree, to which populations were structured, according to geographical location and host-plant association. 83 individuals were sampled from 27 locations ranging from southern California to western Idaho. Restriction site variation was screened by digestion with 11 endonucleases followed by Southern blotting, 38 restriction site positions were mapped by double digests. Parsimony analysis of the resulting 12 haplotypes indicated humungous geographical structuring of populations: (1) most populations were monomorphical for haplotype; (2) haplotypes from California and the Pacific-Northwest (Oregon, Washington and Idaho) formed strongly robust monophyletic groups. Haplotype diversity (π) was five times as high in California as in the Pac-NW (0.011 vs. 0.002), suggesting a recent single origin of all of the latter populations from a source other than Northern California populations.

Introduction

Adaptation of one species to the other members of its community is a primary mechanism by which biodiversity, the diversity of species and interactions, is generated. Our understanding of how communities come to be filled with species performing a variety of functions in a complex web of relationships has been somewhat constrained by the tendency for studies of ecological interaction to focus on one of two ends of a spectrum of hierarchies: (1) studies of paired local populations of interacting species, or (2) studies of the distribution of a type of ecological interaction among species or higher taxa, preferably with reference to their phylogenetic relationship. Concentration on the ends of the hierarchy obscures the fact that species differences in ecological interactions are the result of a process of geographic differentiation. When considered, widespread species often illustrate a shifting mosaic of

interactions among populations. In order to connect the diverse processes of local interactions with their result, macroevolutionary patterns, evolutionary ecologists need to reconstruct how ecological interactions have evolved among populations of widespread, ecologically diverse species. Intraspecific phylogeography, the analysis of phylogenetic relationships and distributions of alleles within a species can provide the historical template upon which the geographic mosaic may be laid. Such phylogeographic studies using variation in mitochondrial DNA (hereafter mtDNA) haplotypes are increasingly available for the study of one of the most historically active areas of research on ecological interactions, the relationships of herbivores with their plant hosts. These studies have proven effective in providing the framework for evaluating arguments about plant-herbivore coevolution, including the relative importance for diversification of parallel cladogenesis and host shifts in diversification (Brown, 128).

Phylogeographic studies are most useful when embedded in a research program that includes both lower (population) and higher (phylogenetic) level studies of ecological interaction. Here we report on a phylogeographic study of a widespread, ecologically diverse herbivore, *Greya politella* (Lepidoptera: Prodoxidae) which is well studied at both of these levels. *Greya politella* populations are found in western North America from southern CA to the Pac NW, and from the coastal range to the Rockies. The females insert eggs into the ovaries of one of several species of the genus *Lithophragma* (Saxifragaceae) or *Heuchera grossulariifolia* (Saxifragaceae), and the larvae eat some of the seeds. *Greya politella* populations vary geographically in host association (Table 1), and, as in other *Greya* species, populations are local host-plant specialists (Thompson 1987, 1994, Davis et al. 1992). Since oviposition occurs through the corolla of the flower, females may act as a pollinator of their hosts as well as a seed parasite, although the importance of *G. politella* as a pollinator may vary geographically with the abundance of co-pollinators (Thompson and Pellmyr, 1992).

The discovery that *Greya politella* is a pollinating seed parasite is made more compelling by its close relationship to true yucca moths (*Tegeticula* and *Parategeticula*), which

are obligate mutualists with their yucca hosts (Riley 1892, Powell & Mackie 1966, Davis 1967, Aker & Udovic 1981). Phylogenetic studies of the prodoxid genera (Brown et al. 1994a) indicate that *Greya* species constitute a sister clade to prodoxid genera associated with yuccas and other members of the Agavaceae. This study and a phylogenetic study of *Greya* species (Brown et al., 1994b) have together provided the framework for understanding the evolution of behavioral and ecological characters involved in this specialized interaction (Pellmyr and Thompson 1992, Pellmyr et al. submitted). These studies, however, also provide the framework for understanding the role host-plant association has played in the process of diversification.

Materials and Methods

Total DNA was extracted from each specimen using the protocol of Harrison et al. (1987). The DNA was then digested with one of 11 restriction endonucleases (XbaI, PstI, HindII, EcoRV, BamHI, EcoNI, SstI, BclI, XhoI, MspII and BstNI) and the resulting fragments separated by size via electrophoresis on 0.8% agarose gels according to the protocols of Maniatis et al. (1982). Enzymes were chosen because they exhibited restriction site variation in mtDNA among multiple species of *Greya* (J. Brown, unpublished data). The digested fragments were then transferred to a Zetabind filter using the alkaline transfer process (Stellweg and Dahlberg 1980). Due to the small size and limited number of specimens of *Greya politella*, purified gypsy moth (*Lymantria dispar*) mtDNA was used as a heterologous probe. The *L. dispar* mtDNA was hybridized to the filter after radiolabelling with ³²P (using the random priming method of Feinberg and Vogelstein [1983]) and fragments visualized with autoradiography. The sizes of fragments (\pm 50bp) were estimated by comparing their migration distances with those of a one kilobase ladder size standard, the homology of each restriction site was determined by mapping relative positions using double digests. After all the data was obtained, it was entered into the computer and then analyzed.

Results

The data is shown in Figures 1-3 and Table 1. The mtDNA molecule of *G. politella* consisted of approximately 15.3 kilobases, which is typical for *Greya* species in general (Brown, unpublished data). Several individuals from the southern Oregon populations had genome sizes 300-900 bp larger, caused by variation in a single region (between map units 10 and 14). This region is variable in size in other Lepidoptera (S. Bogdanowicz, A. Brower, personal comm.) and may contain the "A-T rich" or control region of the genome.

The eleven restriction enzymes produced 38 restriction sites, of which 22 were variable and 13 phylogenetically informative. A total of 12 haplotypes were characterized, including that of the outgroup *G. enchrysa*. Assuming no bias in restriction site gains and losses, PAUP found 22 MP trees of relationship among these haplotypes (one of which is shown in Figure 2; strict consensus tree shown in Figure 3).

We found strong concordance between phylogenetic and geographic divisions among the *G. politella* haplotypes; haplotypes C1-C7 were found only in California and haplotypes W1-W4 only in the Oregon, Washington and Idaho (hereafter, Pac-NW) populations (Figures 1 and 4). In addition, populations were usually monomorphic; when polymorphic, multiple haplotypes differed by a single restriction site. Nucleotide diversity (π) was estimated at 0.0156 for all *G. politella* populations. There was, however, a five-fold greater nucleotide diversity among California populations compared with Pac-NW populations (0.0108 vs. 0.0020); 49 of 56 individuals sampled from the latter populations carried the identical haplotype, W1. Average sequence divergence among the California haplotypes was 1.4% compared with 0.8% among Pac-NW haplotypes, whereas the average between region divergence was 3.2% .

Discussion

The most striking phylogeographic pattern is the rather extensive difference between haplotypes in California and the Pac-NW; haplotypes from each region were a well-defined monophyletic group sharing 2-3 non-homoplasious changes (Figure 1), and average estimated

sequence divergence between haplotypes from the two regions was 3.2% (\pm 1.1 S.D.) (Table 3). This is probably an overestimate of sequence divergence, since enzymes producing no variation were eliminated early in the screening process. For comparison, the estimated divergence based on restriction sites between haplotypes W1 (Smoot Hill, WA) and C5 (Sequoia, CA) is 4.5%, whereas an estimated sequence divergence of 2.9% was found in a comparison of a 780 bp sequence from the mitochondrial cytochrome oxidase I and II genes of the same haplotypes (Brown et al. 1994b). If one applies the commonly used molecular clock estimate for mitochondrial genes of 2-2.3% sequence divergence/million years in these genes (see Brower 1994 for evidence for insects), the split between California and Pac-NW populations may have occurred from 1.4-1.6 million years ago, i.e. during the Pleistocene glaciations. Repeated southern advances of the Cordilleran ice sheet during this period and concomitant changes in climate and host-plant distributions may have forced the ancestors of the current California and Pac-NW populations into separate refugia. The dearth of nucleotide diversity in the Pac-NW populations suggests that a population bottleneck occurred during colonization of the area following the retreat of the ice shield during the last 20,000 years (although a "selective sweep" could also explain low haplotype diversity). The Pac-NW haplotypes' distant phylogenetic relationships from northern California genotypes (C6-7) is inconsistent with expansion of these populations gradually northward into the Pac-NW; genetic analysis of newly discovered *G. politella* populations from Colorado (O. Pellmyr unpublished data) and eastern Idaho and western Montana (Thompson, unpublished data) may provide more evidence for the location of the ancestral sources for Pac-NW populations.

The large number of host species used by *G. politella* and the strong phylogeographic structure apparent in host use makes it possible to consider the historical sequence of host plant use in this species. Strong evidence of the sequence of host shifts is most obvious when haplotypes from populations associated with one (derived) host arise from within a cluster of haplotypes from another (ancestral) host. A number of such patterns have been found as

phylogenetic studies increasingly included multiple haplotypes of each species (e.g. for phytophagous insects, Sperling 1993, Brown et al. 1994b, Sperling and Harrison 1994, Brown et al. in press, Funk et al. in press). The lack of phylogenetic resolution among the haplotypes within each of the two *G. politella* clades makes it impossible to reconstruct the sequence of host shifts within these groups of populations with confidence. Nevertheless, populations in both California and Washington use the widespread species *Lithophragma affine*, which is also the only host plant shared between populations in the two clades. (This species hybridizes readily with the more northerly distributed *L. parviflorum*; and it is possible that the two species are actually extremes of a single entity with clinal variation from north to south [Thompson, unpublished data].) These facts are consistent with the hypothesis that *L. affine/parviflorum* is the ancestral host for *G. politella*, with shifts having occurred onto derived hosts within each region. If *L. affine/parviflorum* is the ancestral host, a radiation has occurred in California onto a group of closely related (Soltis et. al 1992) California endemics, *L. bolanderi*, *L. heterophyllum* and *L. cymbalaria*. In the Pac-NW populations, shifts have occurred onto *L. tenellum*. in Washington and onto the saxifrage species *Heuchera grossulariifolia* in Idaho, in parts of which the latter species is broadly sympatric with *L. parviflorum*. The fact that Pac-NW populations sharing the most common haplotype (W1) are found on at least 4 different host species suggests the recent origin of these shifts.

The establishment of an historical framework at this large-scale geographic level complements our understanding of the role of host association in diversification in the genus as a whole. These data, which suggest that populations are radiating onto available hosts in different parts of the geographic range, support Brown et al.'s (1994b) conclusion from haplotype relationships in other *Greya* species that host shifts have played a significant role in diversification in the genus as a whole. Knowing the direction of host shifts may also be critical in testing hypotheses concerning the role of co-pollinators in influencing reciprocal adaptation

of plant and pollinator (Thompson and Pellmyr 1994). This study provides the large-scale template for continuing studies of population structure at more local geographic scales.

Acknowledgments

I big shout out to all the members of my house who kept me in beer while I wrote this paper.

Thanks as well to my cat, Bob.

References

- Brown JM, Pellmyr O, Thompson JN, Harrison RG (1994b) Phylogeny of *Greya* (Lepidoptera: Prodoxidae) based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. *Molecular Biology and Evolution*, **11**,128-141.
- Brown JM, Abrahamson WG (in press) Mitochondrial DNA phylogeography of host races of the goldenrod ball gallmaker, *Eurosta solidaginis* (Diptera: Tephritidae). *Evolution*.
- Davis DR (1967) A revision of the moths of the subfamily Prodoxinae (Lepidoptera: Incurvariidae). *U.S. Natural History Museum Bulletin* **255**, 1-170.
- Davis DR, Pellmyr O, Thompson JN (1992) Biology and systematics of *Greya* Busck and *Tetragma* n. gen. (Lepidoptera: Prodoxidae). *Smithsonian Contributions to Zoology* **524**, 1-88.
- Brower, AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly, *Heliconius erato*, inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences USA*, **91**, 6491-6495.
- Brown JM, Pellmyr O, Thompson JN, Harrison RG (1994a) Mitochondrial DNA phylogeny of the Prodoxidae (Lepidoptera: Incurvarioidea) indicates a rapid ecological diversification of the yucca moths. *Annals of the Entomological Society of America*, **87**, 795-802.
- Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry*, **132**, 6-13.

- Funk DJ, Futuyma DJ, Orti G, Meyer A (in press) A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): new evidence from mitochondrial DNA. *Evolution*.
- Harrison RG, Rand DM, Wheeler WC (1987) Mitochondrial DNA and variation in field crickets across a narrow hybrid zone. *Molecular Biology and Evolution*, **4**, 144-158.
- Maniatis T, Fritsch EF, Sambrook J (1982). *Molecular Cloning*. Cold Spring Harbor Laboratory, MA.
- Nei M, Miller JC (1990) A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. *Genetics*, **125**, 873-879.
- Pellmyr O, Thompson JN, Brown JM, Harrison RG (submitted) Evolution of pollination and mutualism in the yucca moth lineage. *American Naturalist*.
- Stellwag EJ, Dahlberg AE (1980) Electrophoretic transfer of DNA, RNA, and protein onto diazobenzylmethyl(DMB)-paper. *Nucleic Acids Research*, **8**, 299-317.
- Swofford DL (1993) *PAUP:Phylogenetic Analysis Using Parsimony, version 3.1.1*. Computer program distributed by the Illinois Natural History Survey, Champaign, IL.
- Thompson JN (1987) Variance in number of eggs per patch: oviposition behaviour and population dispersion in a seed parasitic moth. *Ecological Entomology*, **12**, 311-320.
- Thompson JN (1994) *The Coevolutionary Process*. University of Chicago Press, Chicago
- Thompson J N, Pellmyr O (1992) Mutualism with pollinating seed parasites amid co-pollinators: constraints on specialization. *Ecology* **73**, 1780-1791.
- Soltis DE, Soltis PS, Thompson JN (1992) Chloroplast DNA variation in *Lithophragma* (Saxifragaceae). *Systematic Botany*, **17**, 607-619.
- Sperling FAH (1993) Mitochondrial DNA phylogeny of the *Papilio machaon* species group (Lepidoptera: Papilionidae). *Memoirs of the Entomological Society of Canada*, **165**, 233-242.
- Sperling FAH, Harrison RG (1994) Mitochondrial DNA variation within and between species of the *Papilio machaon* species group of swallowtail butterflies. *Evolution* **48**, 408-422.
- Sperling FAH, Hickey DA (1994) Mitochondrial DNA sequence variation in the spruce budworm species complex (*Choristoneura*: Lepidoptera). *Molecular Biology and Evolution*, **11**, 656-665.

Figure Legends

Figure 1. Restriction site map for *Greya politella* haplotypes used this study. Units are kilobases with reference to the conserved pattern of BstNI.

Figure 2. One of 22 MP haplotype networks for *Greya politella* [length = 30, C.I. (excluding uninformative) = 0.619]. Changes in characters (see Table 2) were inferred using PAUP 3.1.1 assuming accelerated transformation with the *G. enchrysa* haplotype as the outgroup. Homoplasious characters on this tree are indicated in bold.

Figure 3. Strict consensus of all MP trees of *Greya politella* haplotypes assuming equal (n=22), 2:1 (n=8) and 5:1 (n=8) weights for gains and losses.

Figure 4. Geographic distribution of haplotypes and host-plant associations of sampled *Greya politella* populations.