

Mitochondrial DNA variation and Haldane's rule in the *Papilio glaucus* and *P. troilus* species groups

FELIX A. H. SPERLING

Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, NY 14853-2701, U.S.A.

Variation in mitochondrial DNA (mtDNA) was surveyed, using restriction endonucleases, in all species of the *Papilio glaucus* and *P. troilus* groups (Lepidoptera: Papilionidae). Phylogenetic and distance relationships of mtDNA generally confirmed traditional species limits in the two species groups and compared favourably with a prior survey of their allozymes. The most notable exceptions were *P. rutulus* and *P. eurymedon*, which share their mtDNA, yet maintain numerous morphological and ecological distinctions in sympatry. In organisms with heterogametic females, like the Lepidoptera, the deficiencies of hybrid females that are expected on the basis of Haldane's rule should lead to congruence of mtDNA variants with species boundaries. In the *P. glaucus* group, the extent of mtDNA differences between species pairs generally coincided with the extent of the Haldane effect, as well as with the presence of major species differences on sex chromosomes (X effect).

Keywords: allozymes, introgression, phylogeny, sex linkage, speciation.

Introduction

Mitochondrial DNA (mtDNA) has become one of the most commonly employed markers for determining genetic relationships among individuals and species (Avice *et al.* 1987; Harrison, 1991). Its generally maternal inheritance, simple structure, small size, rapid evolution and ease of isolation have made the molecule particularly amenable to population genetic and phylogenetic analysis (Avice, 1989, 1991). However, as with any single cluster of tightly linked genes, the phylogeny of mtDNA may not correspond to the average gene phylogeny of the organisms involved. It remains important to establish the effectiveness of mtDNA as a phylogenetic marker, and to investigate conditions under which variation in the molecule may fail to indicate species boundaries.

Since mitochondrial DNA is almost exclusively maternally inherited (Avice, 1991; but see Zouros *et al.*, 1992), elimination of hybrid females should lead to congruence of species boundaries and the distribution of mtDNA variants. Some animals, such as butterflies and birds, show higher rates of sterility or inviability in

F₁ females than in F₁ males and this phenomenon is correlated with the fact that females are the heterogametic sex in these organisms (Haldane, 1922; Coyne & Orr, 1989; Coyne *et al.* 1991; but see Read & Nee, 1991). When the Haldane effect is strong (F₁ females are substantially less viable or fertile than males), and other biological factors like selection are equal, the distribution of different mtDNA haplotypes should conform better to species boundaries in zones of hybrid interactions than should most nuclear genes.

The X effect, which Coyne & Orr (1989) have defined as a disproportionate tendency for genes that are important to hybrid sterility or inviability to occur on sex chromosomes, is correlated with the Haldane effect. The mechanism leading to the Haldane effect or X effect remains uncertain (Coyne *et al.*, 1991; Frank, 1991). Both effects may relate to the fact that sex-linked alleles are more exposed to selection, causing differences to accumulate faster than for autosomal alleles (Charlesworth *et al.*, 1987; Coyne & Orr, 1989). If the Haldane effect and X effect are real and associated with differential selection against individuals of the heterogametic sex, then in species with heterogametic females both effects should be correlated with the presence of mtDNA differences between species.

Present address: Department of Biology, Gendron Hall, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5.

I investigated mtDNA variation in swallowtail butterflies of the *Papilio glaucus* group (six species) and *Papilio troilus* group (three species), all of which are restricted to North America. Morphological comparisons among members of the *P. glaucus* species group were carried out by Brower (1959a), and Hagen *et al.* (1992) have elevated *P. canadensis* to species status. Allozymes of both species groups were analysed phylogenetically by Hagen & Scriber (1991). In the present study, a survey of restriction site variation in mtDNA of the *P. glaucus* and *P. troilus* species groups was used to address two questions: (i) do mtDNA differences occur between species and are they correlated with the Haldane effect or X effect? (ii) how well does the mtDNA phylogeny coincide with prior phylogenetic hypotheses, particularly those of Hagen & Scriber (1991)?

Methods

One or a small number of adult swallowtail butterflies were collected for each species and kept frozen at -80°C until use. Mitochondrial DNA restriction sites were mapped on a total of 31 specimens representing different localities or species (Table 1). Each haplotype

Table 1 Specimens examined, grouped by taxon. (CA = California, FL = Florida, WA = Washington)

Taxon (*n*). Locality 1 (haplotype); Locality 2 (haplotype);...

<i>P. glaucus</i> (4).	Potomac, Maryland (G1); Great Falls, Maryland (G1); Ocala State Forest, FL (G1); Stone Co., Arkansas (G2)
<i>P. alexiars garcia</i> (1).	Tamaulipas, Mexico (G3)
<i>P. canadensis</i> (5).	Richford, New York (G4); Bragg Creek, Alberta (G4); Fairbanks, Alaska (G5); Madison, Wisconsin (G6); Black Hills, South Dakota (G7)
<i>P. rutulus</i> (6).	Orcas Isl., WA (R1); Alpine Co., CA (R1); Columbia R., Oregon (R2); Mono Co., CA (R2); Nevada Co., CA (R2); San Francisco, CA (R3)
<i>P. eurymedon</i> (5).	Alpine Co., CA (R4); Mono Co., CA (R5); Orcas Island, WA (R6); Nevada Co., CA (R7); Los Angeles Co., CA (R8)
<i>P. multicaudatus</i> (2).	Black Hills, South Dakota (MU1); Okanogan Co., WA (MU2)
<i>P. troilus ilioneus</i> (2).	Ocala State Forest, FL (T1); Highland St. Park, FL (T1)
<i>P. troilus troilus</i> (2).	Blue Ridge, Virginia (T2); Fayette Co., Tennessee (T2)
<i>P. palamedes palamedes</i> (2).	Ocala State Forest, FL (PA1); Highland State Park, FL (PA2)
<i>P. palamedes leontis</i> (1).	Tamaulipas, Mexico (PA3)
<i>P. pilumnus</i> (1).	Tamaulipas, Mexico (PI)

was designated with a separate code, to distinguish mtDNAs from the species that contain them. Voucher specimens of the wings and/or abdomen of each specimen are deposited as lot no. 1204 in the Entomology Collection of Cornell University.

Total DNA was extracted from the thoraces of single butterflies using the protocol of Harrison *et al.* (1987). Small aliquots (generally 1/100th of the original DNA extracted) were digested separately with 17 restriction endonucleases: *Bam*HI, *Bcl*II, *Bgl*III, *Bst*BI, *Bst*EII, *Bst*NII, *Clal*, *Eco*RI, *Hae*III, *Hind*III, *Msp*I, *Pst*I, *Pvu*II, *Sca*I, *Sst*I, *Xba*I and *Xho*I. Fourteen of the enzymes recognize sequences of 6 bp, *Hae*III and *Msp*I recognize sequences of 4 bp and *Bst*NI recognizes the sequence CC(A/T)GG. DNA fragments were separated in 0.8 per cent agarose gels and transferred to nylon filters with 0.4 N sodium hydroxide. Pure mtDNA was separated from *Papilio polyxenes* total DNA by differential centrifugation in a CsCl gradient, and then used to probe for mtDNA on the nylon filters. *P. polyxenes* mtDNA was labelled with ^{32}P by random priming, hybridized to DNA fragments on nylon filters, and visualized by autoradiography.

Fragment sizes were estimated by comparison to lanes with a one kilobase size standard (New England Biolabs) and the positions of sites were determined by double digestions. Sequence divergence was estimated with the iterative estimation technique of Nei & Tajima (1983). Phylogenetic trees were obtained by analysing presences and absences of restriction sites as unordered characters using PAUP 3.0q (Swofford, 1991).

Results

Haplotypes and genetic divergences

A total of 23 different haplotypes was found among the 32 maternal lineages surveyed. The seven most divergent haplotypes are shown in Fig. 1 and minor haplotypes are described in the figure legend. Except for *P. pilumnus*, which had an insertion of 0.8 kilobases, all haplotypes had a size of about 15.2 kilobases. No size variation or site polymorphism was detected within individuals. A total of 102 restriction sites was detected, with a range of 42–50 per individual. The mean number of sites was 46.6 per individual, which represents about 1.7 per cent of the mtDNA genome.

In the *P. glaucus* group, four groups of haplotypes had divergences of 1 per cent or less. These corresponded to the mtDNAs of: 1. *P. glaucus* + *P. alexiars*; 2. *P. canadensis*; 3. *P. rutulus* + *P. eurymedon*; and 4. *P. multicaudatus*. One haplotype of *P. rutulus* (R3) was more similar to two mtDNAs of *P. eurymedon* (R4 and

Fig. 1. Linearized mtDNA restriction site maps of seven major haplotypes in the *P. glaucus* and *P. troilus* species groups. Haplotype designations are on the left margin. Sites that are conserved in all haplotypes are shown at the top of the figure. Letter code for restriction enzymes is as follows: a = *Bam*HI, b = *Bst*BI, c = *Cla*I, d = *Hind*III, e = *Eco*RI, f = *Bcl*I, g = *Bgl*II, h = *Hae*III, k = *Sca*I, m = *Msp*I, n = *Bst*NI, p = *Pst*I, s = *Sst*I, t = *Bst*EII, v = *Pvu*II, x = *Xba*I, and no code for *Xho*I. Minor haplotypes are characterized as follows:

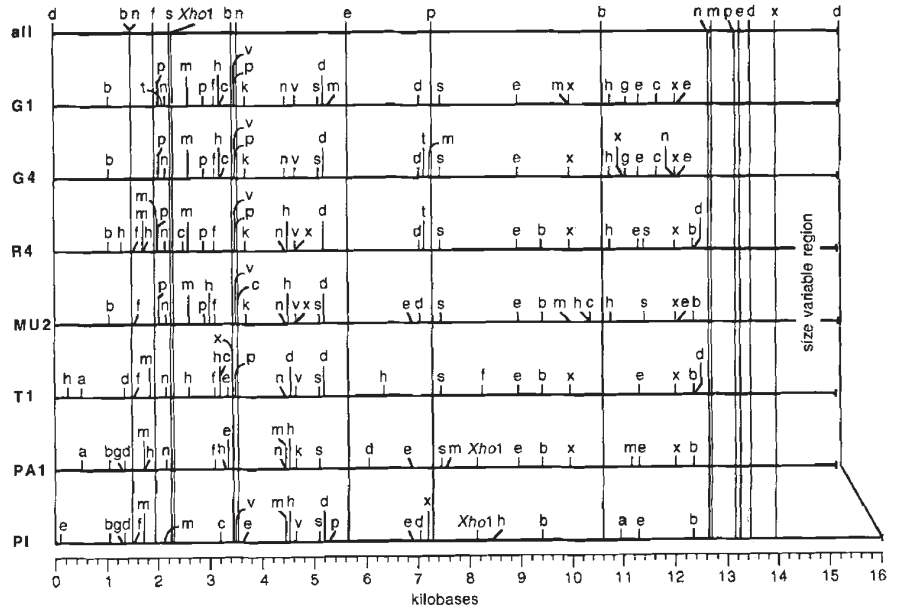
G2 = G1 + c10.85 - p2.90;
 G3 = G1 + h3.6 + d4.15;
 G5 = G4 - x12.0;
 G6 = G4 - f3.1 - m7.25;
 G7 = G4 - e12.05 - m7.25 - m11.15;
 R1 = R4 - b12.35;
 R2 = R4 - b12.35 - p2.05 + n4.4;
 R3 = R4 + m6.9 - v4.65;
 R5 = R5 + m5.3;
 R6 = R4 + m7.05 - v4.65;
 R7 = R4 + g11.05;
 R8 = R4 - f1.55 - v4.65;
 MU1 = MU2 + a2.1;
 T2 = T1 + h10.3 + s1.6;
 PA2 = PA1 + g10.4 - b12.35 + h10.3;
 PA3 = PA1 - b12.35 + h10.3 + h12.35 -
 + m12.4.

R6) than it was to the other haplotypes found in *P. rutulus* (R1 and R2). All mtDNAs of these two species showed divergences of 1 per cent or less. *P. glaucus* and *P. alexiaries* mtDNAs were also very similar (0.4–0.8 per cent). Divergences between all other pairs of species were more substantial (1.9–5.1 per cent).

In the *P. troilus* group, mtDNAs of separate subspecies were very similar (0.4% for *P. troilus* and 0.6–0.8 per cent for *P. palamedes*). Haplotypes of different species of the *P. troilus* group showed much greater divergences than within the *P. glaucus* group, with divergences among the mtDNAs of *P. troilus*, *P. palamedes* and *P. pilumnus* ranging from 7.2 to 9.6 per cent.

Genetic network

When restriction sites were considered as unordered characters for phylogenetic analyses, a single most parsimonious network was found by a branch and bound search of the full set of 23 haplotypes (Fig. 2). The consistency index of this tree was 0.67, excluding uninformative characters.



The gene network supported the separation of mtDNA haplotypes found in *P. canadensis* from a clade including mtDNAs of *P. glaucus* and *P. alexiaries*. Within *P. g. glaucus*, the Florida populations are sometimes referred to as a separate subspecies, *P. g. australis* (Scriber *et al.*, 1991); however, the G₁ haplotype was found in both Maryland and Florida. Within *P. canadensis*, Alaskan and Yukon populations are also sometimes recognized as a separate subspecies, *P. g. arcticus*. The phylogenetic relationship of the G₅ haplotype, from Alaska, showed only a single restriction site difference from the G₄ haplotype, which was found in Alberta and New York.

Each of the five *P. eurymedon* specimens had a different haplotype, and the six *P. rutulus* specimens had three haplotypes. Interestingly, the mtDNAs of *P. rutulus* and *P. eurymedon* appeared to form a single clade, but the mtDNAs of neither *P. rutulus* nor *P. eurymedon* were monophyletic.

mtDNA versus allozymes

In order to facilitate comparison between my findings and those of Hagen & Scriber (1991), the haplotype dataset and allozyme dataset were condensed to 11 groups corresponding to taxonomic species or major subspecies (Fig. 3). Genetic distance comparisons of mtDNA were accomplished by averaging values within the 11 taxonomic groups. In some cases these groups did not correspond to monophyletic mtDNA lineages;

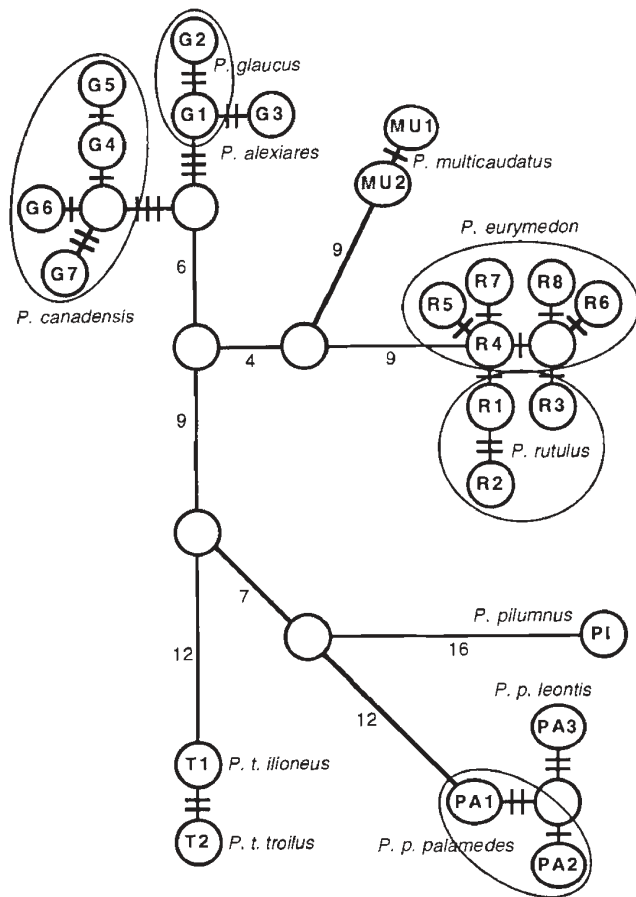


Fig. 2. Parsimony network of mtDNA haplotypes. Codes in circles refer to haplotypes of specimens surveyed (listed in Table 1). Numbers or bars on branches indicate numbers of restriction site changes (apomorphies). Branch lengths are adjusted in proportion to numbers of site changes. Species identifications of butterflies are indicated beside their haplotypes.

the mtDNAs of *P. glaucus*, *P. eurymedon*, *P. rutulus*, and *P. p. palamedes* were paraphyletic (Fig. 2). Parsimony analysis using PAUP 3.0q (Swofford, 1991) was performed on all haplotypes in the *P. glaucus* and *P. troilus* groups. Figure 3 includes a consensus tree based on heuristic searches of 500 bootstrap resamplings of the 23 haplotypes, with restriction sites considered as unordered characters. The consensus tree was rooted at the midpoint between the two most distant nodes.

The cladogram of allozyme data was redrawn from Hagen & Scriber's (1991) figure, whereas the UPGMA phenogram of Nei's distances was recalculated from their table of Nei's genetic identities. The topology of the *P. glaucus* group in the phenogram in Fig. 3 differs from the branching pattern depicted in Hagen &

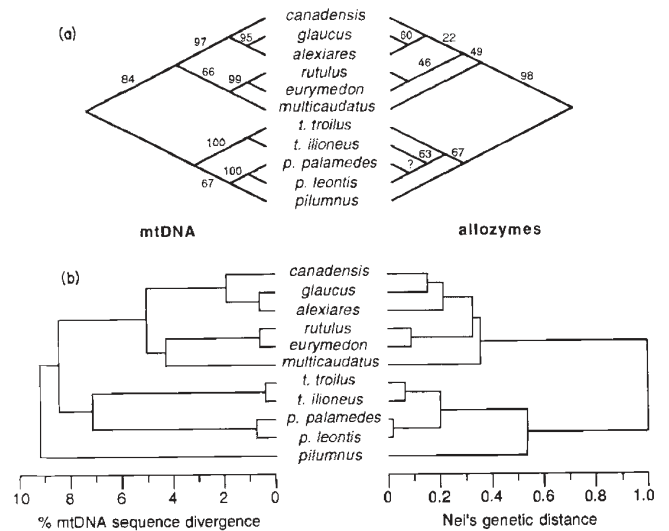


Fig. 3 Comparison of trees obtained from analysis of mtDNA, on the left, versus allozymes, on the right. Cladograms (a) are at the top, with numbers along branches referring to percent bootstrap support for particular groupings. UPGMA phenograms (b) are at bottom. The phenogram of mtDNA genetic divergences is calculated as *d* of Nei & Tajima (1983). The allozyme cladogram is redrawn from Hagen & Scriber (1991) and the phenogram is recalculated from their table of Nei's standard genetic identities.

Scriber's (1991) phenogram, and now shows a closer correspondence to their cladogram.

The mtDNA and allozyme cladograms differed at three points. The mtDNA phylogeny weakly supported a sister group relationship for *P. multicaudatus* with *P. rutulus* + *P. eurymedon*, rather than branching off basally below the rest of the *P. glaucus* group. The mtDNA phylogeny also weakly supported a sister group relationship for *P. pilumnus* with *P. palamedes*, rather than with both *P. palamedes* and *P. troilus*. There was strong support from mtDNA for the monophyly of *P. troilus*, without any paraphyletic relationship with *P. palamedes*. Based on bootstrapping analyses, the branching pattern of the mtDNA phylogeny was, in general, much better supported than was the branching pattern of the allozyme phylogeny.

The UPGMA phenograms generated from pairwise genetic distances also showed good correspondence. The phenogram of mtDNA distances differed from the mtDNA cladogram in that *P. pilumnus* was basal to both the *P. troilus* and *P. glaucus* groups. The allozyme phenogram showed better agreement with mtDNA with respect to the distance relationships of *P. troilus* and *P. palamedes*, but poor agreement within the *P. glaucus* group.

Discussion

mtDNA and species boundaries

Most traditional species in the *P. glaucus* and *P. troilus* groups are characterized by discrete clusters of mtDNA haplotypes. *P. rutulus* and *P. eurymedon* were exceptions, even though there appears to be no question that they are separate species. *P. eurymedon* adults have a pale yellow coloration that distinguishes them from all other *P. glaucus* group species. This colour trait reduces courtship of *P. eurymedon* females by males of *P. multicaudatus* (Brower, 1959b). It is reasonable to expect that the same is true of *P. eurymedon* and *P. rutulus*, but the necessary studies have not yet been done. *P. eurymedon* and *P. rutulus* also have different male genitalia (Brower, 1959a) and their first instar larvae differ in their survivorship on several host plants (Scriber *et al.*, 1991). The two species overlap across virtually all of their range and viable hybrid adults of both sexes can be produced by hand pairing (Scriber *et al.*, 1990), but very few natural hybrids have been found (Wagner, 1978).

Relationships among the mtDNA haplotypes of *P. rutulus* and *P. eurymedon* suggest that either: (i) mtDNA polymorphism has been retained through the speciation of *P. rutulus* and *P. eurymedon*; or (ii) at least two mtDNA introgression events have occurred between *P. rutulus* and *P. eurymedon*, followed by elimination of the original mtDNAs in one species. Allozyme differences between *P. eurymedon* and *P. rutulus* are small (Fig. 3), and neither alternative can easily be excluded.

P. glaucus and *P. alexiaries* also have very similar mtDNA. *P. glaucus* overlaps a substantial part of the range of *P. alexiaries* in northern Mexico but the extent to which the species maintain their integrity along the zone of contact seems uncertain (Beutelspacher & Howe, 1984). In contrast, the extent of allozyme differences between them is greater than between *P. glaucus* and *P. canadensis* (Fig. 3), indicating significant divergence of nuclear genes.

A partial lack of correspondence between traditional species boundaries and mtDNA haplotype distributions has also been found in insects such as *Drosophila* (Powell, 1983; Aubert & Solignac, 1990), cicadas (Martin & Simon, 1990) and weevils (Boyce, 1990). These examples suggest that it will be interesting to survey the mtDNA of *P. eurymedon*/*P. rutulus* and *P. glaucus*/*P. alexiaries* species pairs throughout their range, to determine whether any older mtDNA lineages can be found and under what conditions they may have been preserved.

mtDNA and the Haldane effect

P. rutulus/*P. eurymedon* and *P. glaucus*/*P. alexiaries* are among the few pairs of closely related *Papilio* species that do not show a Haldane effect (Ae, 1979; Table 2). In contrast, deficiency of females is pronounced in hybrids between *P. glaucus* and the three western species — *P. eurymedon*, *P. rutulus* and *P. multicaudatus*. There is a smaller, but significant, Haldane effect between *P. glaucus* and *P. canadensis* (Hagen & Scriber, 1993).

In hybrids between *P. glaucus* and *P. canadensis*, or between *P. glaucus* and the western species, ecologically important differences like diapause and female mimetic wing coloration are located on the sex chromosomes (Hagen & Scriber, 1989). In contrast, one of the most important genetic differences between *P. eurymedon* and *P. rutulus* appears not to be controlled by X-linked genes, since female F₁ hybrids from both crosses between *P. glaucus* and *P. eurymedon* have a wing color that is nearly as yellow as that of *P. glaucus* (West & Clarke, 1988). The correlation between mtDNA differences and Haldane effect supports the expectation that differential elimination of females will lead to conformance of mtDNA differences with species boundaries. The correspondence of both factors with the X effect suggests the possibility that interactions between cytoplasmic factors and X-linked genes are involved in the maintenance of species boundaries in these species (see Virdee & Hewitt, 1992, for an example in grasshoppers). However, mtDNA differences may also be due to selection against combinations of autosomal genes and cytoplasmic traits.

P. multicaudatus is a case that counters the correlation of mtDNA differences with the Haldane effect. Possible natural hybrids between *P. rutulus* and *P. multicaudatus* have been reported (Brower, 1959a; Garth & Tilden, 1986) and preliminary evidence suggests that there is no Haldane effect in crosses

Table 2 Correspondence between mtDNA differences, Haldane effect and X effect in species pairs of the *P. glaucus* group (West & Clarke, 1988; Scriber *et al.*, 1990; Hagen & Scriber, 1993)

	mtDNA differences	Haldane effect	X effect
<i>P. glaucus</i> × <i>canadensis</i>	Yes	Yes	Yes
<i>P. glaucus</i> × <i>rutu./eury./mult.</i>	Yes	Yes	Yes
<i>P. glaucus</i> × <i>alexiaries</i>	No	No	?
<i>P. rutulus</i> × <i>eurymedon</i>	No	No	No?
<i>P. multicaudatus</i> × <i>rutu./eury.</i>	Yes	No	?

between *P. multicaudatus* and either *P. rutulus* or *P. eurymedon* (Scriber *et al.*, 1990). Yet the mtDNA of the two specimens of *P. multicaudatus* examined in this study was very distinct. I suggest that both allozymes and mtDNA indicate genetic divergences are so extensive that a large number of genes now contribute to genetic barriers to introgression, obscuring any early contribution that might have been due to genes on sex chromosomes.

To date, mtDNA differences, Haldane effect and X effect have been studied in only two other species groups of Lepidoptera, including the *Papilio machaon* group (Ae, 1979; Thompson, 1988; Sperling, 1993) and the *Choristoneura fumiferana* group (Harvey, 1985; Sperling & Hickey, unpublished). Both cases show correspondence between all three phenomena. A correlation between distinct mtDNA and the Haldane effect or X effect should hold for other closely related species of organisms in which females are the heterogametic sex, including a variety of vertebrates, invertebrates and even plants (Bull, 1983; Tegelström & Gelter, 1990).

mtDNA and species phylogenies

Unlike mtDNA, allozymes survey a number of parts of the nuclear genome. Hence they can be expected to give a more representative picture of genetic divergence among populations. However, as Buth (1984) has stated, 'there has been no consensus on the appropriate treatment of electrophoretic data in phylogenetic studies, and problems have persisted at a variety of levels'. Hagen & Scriber (1991) encountered some of these problems in their survey of *Papilio* allozymes, including incomplete resolution of alleles and the need for arbitrary cutoffs for converting frequency differences into discrete character states.

In contrast, the abundant, clearly resolved variation in mtDNA, together with the absence of recombination, makes the molecule ideal for phylogenetic analysis. The mtDNA survey distinguished 102 restriction sites, each of which was treated as one character with two states. In comparison, the 26 loci in the allozyme survey were each treated as one character, with 1–10 allelic character states per locus. Bootstrap support was higher for the mtDNA phylogeny than the allozyme phylogeny. However, the fact that mtDNA may not be representative of the rest of the genome is well illustrated by *P. rutulus* and *P. eurymedon*, which appear to share their mtDNA in spite of clear morphological, behavioural and allozyme differences. Nonetheless, the paraphyletic relationship indicated by allozymes for the *P. troilus* subspecies, in spite of strong support for monophyly on the basis of both

morphology and mtDNA, indicates that allozymes may also fail to elucidate relationships at this level.

The question remains: is it more informative to have an unclear picture of a large part of the genome, or a clear picture of a small part of the genome? Judging from a comparison of allozymes and mtDNA against the classical species delineations in the *P. glaucus* and *P. troilus* groups, analysis of mtDNA variation is approximately as effective for determining species limits and relationships as is analysis of a battery of allozyme loci.

Acknowledgements

I greatly appreciate the special efforts made by people who obtained live or frozen swallowtails for me, including M. Ayres, M. Carter, M. Evans, P. Feeny, M. Furr, R. Hoebeke, R. Lederhouse, D. McCorkle, M. Peterson, M. Scriber, A. Shapiro, B. Sperling and C. Yoon. I thank S. Bogdanowicz for advice in the lab, R. Hagen and R. Lederhouse for discussion, and K. Brown, P. Feeny, R. Harrison and D. Hickey for comments on various forms of the manuscript. This study was supported by grants from the National Science Foundation (BSR-8914620 to F. S., BSR-8906707 to R. G. Harrison and BSR-8516832 to P. Feeny).

References

- AE, S. A. 1979. The phylogeny of some *Papilio* species based on interspecific hybridization data. *System. Entomol.*, **4**, 1–16.
- AUBERT, J. AND SOLIGNAC, M. 1990. Experimental evidence for mitochondrial DNA introgression between *Drosophila* species. *Evolution*, **43**, 1192–1208.
- AVISE, J. C., ARNOLD, J., BALL, R. M., BIRMINGHAM, E., LAMB, T., NEIGEL, J. E., REEB, C. A. AND SAUNDERS, N. C. 1987. Intra-specific phylogeography: the mitochondrial DNA bridge between genetics and systematics. *Ann. Rev. Ecol. System.* **18**, 489–522.
- AVISE, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution*, **43**, 1192–1208.
- AVISE, J. C. 1991. Ten unorthodox perspectives on evolution prompted by comparative population genetic findings on mitochondrial DNA. *Ann. Rev. Genet.*, **25**, 45–69.
- BEUTELSPACHER, C. R. AND HOWE, W. H. 1984. *Mariposas de Mexico*. vol. 1. La Prensa Medica Mexicana, S. A., Mexico, D. F.
- BOYCE, T. M. 1990. *Molecular evolutionary genetics of the bark weevils: speciation and mitochondrial DNA evolution*. Ph. D. Thesis, Cornell University.
- BROWER, L. P. 1959a. Speciation in butterflies of the *Papilio glaucus* group. I. Morphological relationships and hybridization. *Evolution*, **13**, 40–63.

- BROWER, L. P. 1959b. Speciation in butterflies of the *Papilio glaucus* group. I. Ecological relationships and interspecific sexual behavior. *Evolution*, **13**, 212-228.
- BULL, J. J. 1983. *Evolution of Sex Determining Mechanisms*. The Benjamin/Cummings Publ. Co., Menlo Park, California.
- BUTH, D. G. 1984. The application of electrophoretic data in systematic studies. *Ann. Rev. Ecol. Syst.*, **15**, 501-522.
- CHARLESWORTH, B., COYNE, J. A. AND BARTON, N. H. 1987. The relative rates of evolution of sex chromosomes and autosomes. *Am. Nat.* **130**, 113-146.
- COYNE, J. A. AND ORR, H. A. 1989. Two rules of speciation. In: Otte, D. and Endler, J. A. (eds) *Speciation and its Consequences*, Sinauer Assoc., Sunderland, Massachusetts, pp. 180-207.
- COYNE, J., CHARLESWORTH, B. AND ORR, H. A. 1991. Haldane's rule revisited. *Evolution*, **45**, 1710-1714.
- FRANK, S. A. 1991. Haldane's Rule: A defense of the meiotic drive theory. *Evolution*, **45**, 1714-1717.
- GARTH, J. S. AND TILDEN, J. W. 1986. *California Butterflies*. University of California Press, Berkeley.
- HAGEN, R. H. AND SCRIBER, J. M. 1989. Sex-linked diapause, color, and allozyme loci in *Papilio glaucus*: linkage analysis and significance in a hybrid zone. *J. Hered.*, **80**, 179-185.
- HAGEN, R. H. AND SCRIBER, J. M. 1991. Systematics of the *Papilio glaucus* and *P. troilus* species groups (Lepidoptera: Papilionidae): inferences from allozymes. *Ann. Ent. Soc. Am.*, **84**, 380-395.
- HAGEN, R. H. AND SCRIBER, J. M. 1993. Sex chromosomes and speciation in tiger swallowtails. In: Scriber, J. M., Tsubaki, Y. and Lederhouse, R. C. (eds) *The Ecology and Evolutionary Biology of the Papilionidae*. Scientific Publishers, Inc., Gainesville, Florida. (in press)
- HAGEN, R. H., LEDERHOUSE, R. C., BOSSART, J. L. AND SCRIBER, J. M. 1992. *Papilio canadensis* and *P. glaucus* (Papilionidae) are distinct species. *J. Lepid. Soc.*, **45**, 245-258.
- HALDANE, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. *J. Genetics*, **12**, 101-109.
- HARRISON, R. G. 1991. Molecular changes at speciation. *Ann. Rev. Ecol. System.*, **22**, 281-308.
- HARRISON, R. G., RAND, D. M. AND WHEELER, W. C. 1987. Mitochondrial DNA variation in field crickets across a narrow hybrid zone. *Mol. Biol. Evol.*, **4**, 144-158.
- HARVEY, G. T. 1985. The taxonomy of the coniferous Choristoneura (Lepidoptera: Tortricidae): A review. In: Sanders, C. J., Stark, R. W., Mullins, E. J. and Murphy, J. (eds) *Recent Advances in Spruce Budworms Research*. Proc. CANUSA Spruce Budworms Research Symposium, Bangor, Maine. Canad. Forestry Service, Ottawa, Ontario, pp. 16-48.
- MARTIN, A. AND SIMON, C. 1990. Differing levels of among-population divergence in the mitochondrial DNA of periodical cicadas related to historical biogeography. *Evolution*, **44**, 1066-1080.
- MORITZ, C., DOWLING, T. E. AND BROWN, W. M. 1987. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. *Ann. Rev. Ecol. System.*, **18**, 269-292.
- NEI, M. AND TAJIMA, F. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. *Genetics*, **105**, 207-217.
- POWELL, J. R. 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 492-495.
- READ, A. AND NEE, S. 1991. Is Haldane's rule significant? *Evolution*, **45**, 1707-1709.
- SCRIBER, J. M., DOWELL, R. V., LEDERHOUSE, R. C. AND HAGEN, R. H. 1990. Female color and sex ratio in hybrids between *Papilio glaucus glaucus* and *P. eurymedon*, *P. rutulus* and *P. multicaudatus* (Papilionidae). *J. Lepid. Soc.*, **44**, 229-244.
- SCRIBER, J. M., LEDERHOUSE, R. C. AND HAGEN, R. H. 1991. Food-plants and evolution within *Papilio glaucus* and *Papilio troilus* species groups (Lepidoptera: Papilionidae). In: Price, P. W., Lewinsohn, T. M., Fernandes, G. W. and Benson, W. W. (eds) *Plant-Animal Interactions: Evolutionary Ecology in Tropical and Temperate Regions*. John Wiley, New York, pp. 341-373.
- SPERLING, F. A. H. 1990. Natural hybrids of *Papilio*: poor taxonomy or interesting evolutionary phenomenon? *Canad. J. Zool.*, **68**, 1790-1799.
- SPERLING, F. A. H. 1993. Mitochondrial DNA phylogeny of the *Papilio machaon* species group (Lepidoptera: Papilionidae). In: Ball, G. E. and Danks, H. V. (eds) *Systematics in Support of Entomology Mem. Ent. Soc. Canad.*, **165**, 233-242.
- SWOFFORD, D. L. 1991. PAUP: *Phylogenetic Analysis Using Parsimony*, Version 3.0 Q. Computer program distributed by the Illinois Natural History Survey, Champaign, Illinois.
- TEGELSTRÖM, H. AND GELTER, H. P. 1990. Haldane's rule and sex biased gene flow between two hybridizing flycatcher species (*Ficedula albicollis* and *F. hypoleuca*, Aves: Muscicapidae). *Evolution*, **44**, 2012-2021.
- THOMPSON, J. N. 1988. Evolutionary genetics of oviposition preference in swallowtail butterflies. *Evolution*, **42**, 1223-1235.
- VIRDEE, S. R. AND HEWITT, G. M. 1992. Postzygotic isolation and Haldane's rule in a grasshopper. *Heredity*, **69**, 527-538.
- WAGNER, W. H., JR. 1978. A probable natural hybrid of *Papilio eurymedon* and *P. rutulus* (Papilionidae) from Idaho. *J. Lepid. Soc.*, **32**, 226-228.
- WEST, D. A. AND CLARKE, C. A. 1987 (1988). Suppression of the black phenotype in females of the *P. glaucus* group (Papilionidae). *J. Res. Lepid.*, **26**, 187-200.
- ZOUROS, E., FREEMAN, K. R., BALL, A. O. AND POGSON, G. H. 1992. Direct evidence for extensive paternal mitochondrial DNA inheritance in the marine mussel *Mytilus*. *Nature*, **369**, 412-414.