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PARTIAL GENETIC ISOLATION BETWEEN *PHYCIODES THAROS* AND *P. COCYTA* (NYMPHALIDAE)

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ABSTRACT. Systematists have been interested in the *Phyciodes tharos* group since Oliver discovered partial hybrid breakdown in crosses between populations of nominal *P. tharos*. The traits so far identified as diagnostic in the group are difficult to characterize genetically, and there are considerable limitations in using them to ascertain the status of genetic isolation. We searched for genetic markers in *P. tharos* and *P. cocyta* which would make these deductions easier. We found notable frequency differences between the taxa in malate dehydrogenase (MDH) and glutamic-oxaloacetic transaminase (GOT). Though significant statistically, these differences can be maintained by partial genetic isolation and an introgression rate of about 1 genome per generation. A review of the published research on the diagnostic traits is consistent with this interpretation, and we suggest that these taxa be considered as subspecies unless further research turns up evidence of genetic isolation. Previous researchers have invoked strong selection to explain the high similarity among populations in allozyme frequencies of *P. tharos*. We find that such similarity can be maintained by a rate of only 7 individuals exchanged among populations each generation without invoking selection, although very weak selection may also be involved. We found what may be a gene duplication in the phosphoglucomutase (PGM) locus that, if verified, may be useful in phylogenetic studies of *Phyciodes*.

Additional key words: gene flow, genetic population structure, phosphoglucomutase, *Phyciodes pascoensis*, species-level systematics.

Over the last 25 years, the *Phyciodes tharos* species group in eastern North America has attracted the attention of biologists interested in systematics and evolution, and new species-level taxonomic divisions have been proposed. We first review the current status of the taxonomy and its biological basis in light of recent theoretical advances, then present the outcome of a genetic analysis that calls into question the existence of sibling species within the group. We then propose an alternative explanation for the maintenance, in a single polytypic *tharos* species, of apparently adaptive differences in the life-history traits that were originally used to delimit sibling species within the *tharos*-group. Though the available data are best interpreted by a single-species model, it is beyond the scope of this paper to solve the taxonomic problem; for that, a closer look at the contact areas will be required. Throughout, our theme is to draw attention to what we believe is the crux of an old and general problem in species-level systematics, that of inferring underlying biological processes from patterns of differentiation in traits within and among populations. Though the presence of differentiation is widely used to infer reproductive isolation, there are other biological processes that can

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maintain geographic differences in traits we use as diagnostic, even in the face of strong gene exchange. We hope to engender a greater awareness among systematists of new methods available, and problems with established methods, for delimiting reproductively isolated species.

CURRENT TAXONOMY

Nomenclature. Oliver (1972, 1979, 1980) discovered patterns of partial hybrid breakdown in crosses between populations of the widespread butterfly *Phyciodes tharos*, and he initially identified these as Type A and Type B population groups. These groups were subsequently reported to be associated with univoltine (Type B) and multivoltine (Type A) life cycles, adult body size differences, coloration differences in late-instar larvae, and subtle differences in adult wing pattern (Oliver 1980). The taxonomy has since been in a state of confusion and flux. Oliver (1980, 1983) considered the northeastern Type B populations to intergrade continuously with the Rocky Mountain populations, to which the name *P. pascoensis* Wright applies. He therefore classified them into two species, *Phyciodes tharos* Drury (=Type A) and *P. pascoensis* Wright (=Type B) on the basis of these differences and their apparent sympatry in parts of Pennsylvania and West Virginia, as he believed that these differences were evidence of reproductive isolation. Meanwhile, Miller and Brown (1981) suggested in a footnote that the older name *P. selenis* (Kirby 1837) was available for the eastern Canadian populations, and this nomenclature was followed by Opler (1992). Scott (1986a) advocated the name *P. morpheus* (Fabr. 1775) as the oldest and, because its type was lost, he restricted its type locality to Nova Scotia, ensuring its applicability to the univoltine populations. This change was problematic. First, Miller and Brown (1981) considered that type to have probably come from the vicinity of New York City, which is within the range of multivoltine *P. tharos* (Shapiro 1974). Second, Scott (1994) has since reported that Fabricius's name was a junior homonym of a hesperiid described by Pallas in 1771, making *morpheus* unavailable. Scott (1994) also found that the original illustration of *P. cocyta* (Cramer 1777), ostensibly from Surinam, agrees rather well with *tharos*-group individuals from northeastern North America, suggesting *cocyta* as the oldest available name for the northern populations. Scott (1994) has now identified the types or has designated neotypes for all the available names. The neotypes include *P. tharos* (Drury) and *P. euclea* (Bergstraesser 1780) collected near New York City, *P. selenis* (Kirby 1837) from Cumberland House, Saskatchewan, and *P. cocyta* (Cramer 1777) from Cape Breton, Nova Scotia. As Scott's efforts seem the most rigorous and complete, we follow his nomenclature here.

Diagnosis. Though the names now appear sorted out, the diagnos-

tic traits and their underlying biology remain controversial, and there is no trait or trait combination generally agreed to be diagnostic. Oliver (1980, 1983) emphasized the number of annual generations as the primary diagnostic trait, with larval coloration and subtle dorsal wing pattern traits also showing consistent differences. Opler and Krizek (1984) added that *P. tharos* males usually have black antennal clubs whereas those of *P. cocyta* males, and both species' females, are usually orange. Scott (1986a, 1994) indicated that orange antennae are not unusual in male *P. tharos* in the northern and western parts of its range, making this character uninformative there, but he added that minor differences were detectable in the shape of a pair of spines on the male genitalia. For distinguishing *tharos* from *cocyta*, Scott (1994) placed greatest emphasis on the male dorsal hindwing pattern, namely the extent to which the median and postmedian orange bands are fused, although Oliver (1976) showed that this trait was influenced by photoperiod. Scott (1994) considers *cocyta* to have both single- and double-brooded populations. All authors agree that considerable variability and overlap exists in the morphological traits of these taxa (Oliver 1980, Opler & Krizek 1984, Scott 1986a, 1994, Opler 1992), and this ambiguity is compounded by the fact that the wing pattern of *P. tharos* is seasonally polyphenic (Oliver 1976). All agree that putative hybrids exist which defy classification into the two types. Nevertheless, range maps for these taxa have been estimated, mainly from pinned specimens, for use in general butterfly guides (Opler & Krizek 1984, Scott 1986a, Opler 1992).

ASSAYING FOR GENETIC ISOLATION

How useful are the diagnostic traits? It has always been difficult to deduce genetic isolation from a suite of morphological and life-history traits, because no body of theory is yet available that allows us to estimate the rate of gene exchange, a biological process, from geographic variation in such quantitative traits, a statistical pattern. This is partly because quantitative traits are usually influenced by environmental as well as genetic factors, and the relative contributions of each are usually unknown. Patterns of variation may well be due to evolutionarily inconsequential environmental variation, spatial as well as seasonal and annual, particularly where phenotypic plasticity is involved as in *Phyciodes*.

Over the last 20 years, our ability to deduce genetic isolation has been greatly informed by theoretical advances in population genetics. These advances came after it was recognized that it is typological, and misleading, to treat hybridizing systems as comprised of interactions among "pure" forms and "hybrids" (Barton & Hewitt 1985). Rather, such systems are more appropriately reduced to the genetic level and investigated on a gene-by-gene and trait-by-trait basis. This approach explicitly accounts for

all degrees of intermediate genotype, yet it still permits taxonomic entities to be reconstructed, tested, and studied empirically when strong and consistent correlations exist among traits (Barton & Hewitt 1985, Barton & Gale 1993). We adopt this perspective throughout.

Three corollaries of this reductionist viewpoint bear further comment. First, the approach permits us to develop quantitative measures of the degree of genetic isolation between taxa (Porter 1990) through models that relate gene flow (or, when taxa are considered, introgression) to degrees of genetic differentiation. This is a significant advance over the qualitative methods used in classical systematics, but it raises a question: How much introgression is enough to qualify two taxa as conspecific? There are three answers plausible along a continuum, one absolute, one practical from a theoretical perspective, and one practical from an operational perspective. One could maintain an absolute criterion that any introgression at all is enough to invalidate a hypothesized species boundary. Porter et al. (1997b) suggest that a low theoretical limit of 10^{-6} individuals per generation might be appropriate, because this is roughly the inverse of the mutation rate, and alleles identical in state might be expected to arise independently in the opposite taxon at about the same rate. However, this is far below the detectable level as measured by current methods, which permit the estimation of introgression levels above only about 10^{-1} genomes per generation (Porter 1990), and this provides a current operational threshold.

As a second corollary, this viewpoint helps to highlight a subtle distinction between the terms genetic isolation and reproductive isolation. Genetic isolation refers simply to the lack of gene exchange and may be directly measured from patterns of genetic differentiation. Reproductive isolation is sometimes used synonymously, but it often contains allusions to reproductive behavior or physiology (cf. Paterson 1993, Lambert & Spencer 1995), and reduced viability or fecundity of hybrids. These mechanisms are a subset of the processes responsible for genetic isolation, but they cannot be quantified from patterns of genetic differentiation. We therefore use genetic isolation as a more precise term for what we believe is appropriate to measure when testing taxonomic hypotheses. Finally, further emphasizing our view that pattern and process should be kept separate in evolutionary studies, we use the terms contact area and hybrid zone in a purely descriptive sense. They are simply places where ranges abut and, in the latter, where hybridization is known to occur, and we include no allusions to the evolutionary processes that produce these geographic patterns. Rather, these processes must be inferred by evolutionary models, preferably made explicit, that relate processes to the genetic and phenotypic patterns we describe.

Abstracted into the mathematics of this gene-by-gene approach is an

important lesson for species-level systematists. It has long been known that natural selection operates to produce local adaptations in ecologically relevant traits, including life-history traits, coloration, etc. These traits are quite likely to be taken as diagnostic at the species- and subspecies-level by systematists, yet such differences are likely to comprise only a small proportion of the genome. Theory shows that it is a mistake to deduce that these differences necessarily indicate genetic isolation in the remainder of the genome (Barton & Bengtsson 1986). The reasoning is subtle, involving quantitative interactions among gene flow, natural selection, and linkage among neutral and selected genes on the same chromosomes. All genes experience the same rate of gene flow because when individuals move among populations, they carry all their genes with them. Complete genetic isolation can result only when neutral genes, themselves unaffected by environmental variation, experience selection barriers indirectly through their correlations with traits under direct selection. These correlations, called linkage disequilibria (though they may arise for reasons other than physical proximity on the chromosome), are strongest for neutral loci closely linked to genes influencing traits under selection, but they are usually negligible for genes on different chromosomes or at opposite ends of the same chromosome. Correlations among all differentiated traits exist in F_1 hybrids from different parental "types," but with further generations of interbreeding, these correlations decay at rates inversely proportional to their linkage to genes influencing selected traits (Wright 1969), and unlinked traits become uncorrelated after about six generations of hybridization (Robbins 1918). It follows that, in order for complete genetic isolation to occur between differentiated, hybridizing populations, either selection must be so strong that the hybrids are completely infertile (or inviable), or a large number of genes under selection must be distributed throughout the genome such that all neutral loci are closely linked to them (Barton and Bengtsson 1986). If these rather stringent criteria are not met, then the initial correlations break down and neutral and adaptive genes will spread via the hybrids to the other taxon. Such genes contribute to the evolutionary history of both taxa, even if strong differentiating selection continues to maintain differences in the diagnostic traits. Such "diagnostic" traits are not markers for genome-wide differentiation, and at best, can be used only for subspecies recognition.

Viewing the current status of the *Phyciodes tharos* and *P. cocyta* problem in this context, several ambiguities arise. The deduction of genetic isolation from the currently available biological information has tacitly entailed the assumptions that: (i) environmental variation has not appreciably influenced the few diagnostic wing pattern traits that have been identified; (ii) the quantitative traits of body size and voltinism, and per-

haps adult wing pattern, larval coloration, male genital morphology, and suspected pheromone differences (Scott 1986b), are influenced by strong selection; and (iii) the genes controlling variation in these traits and any others producing partial hybrid breakdown comprise a large proportion of the genome. The third assumption was partially refuted by Oliver (1979), who concluded that the partial hybrid breakdown was likely to be due to the action of only a small number of loci. Alternatively, it has been suggested that genetic isolation might be enforced by the differing phenologies of these taxa (Scott 1986a), such that they rarely have the opportunity to interbreed. This hypothesis was not supported by the data in Oliver (1980), who showed that when environmental conditions are held constant, as they would be where the two taxa occur sympatrically and have the opportunity to interbreed, the eclosion patterns following larval diapause of the two taxa are quite similar. The current taxonomy clearly stands on a poor foundation and requires, in addition to better data, greater attention to the logic and methods we use as species-level systematists to infer underlying processes from superficial patterns.

In this article, we report the results of an electrophoretic survey of metabolic enzyme variation undertaken to search for single-locus genetic markers which may be useful in the study of genetic isolation in the *Phyciodes tharos* group. The advantage of using such markers is that a considerable body of theory is available with which to estimate the rates of genetic exchange among populations (Slatkin 1987). The estimation of gene exchange rates between *P. tharos* and *P. cocyta* permits an independent empirical test of the hypothesis that these taxa are genetically isolated (Porter 1990, Porter & Geiger 1995). Throughout the article, we adopt the literary convenience and current taxonomic practice of referring to these taxa as *P. tharos* and *P. cocyta*, without implying any conclusion about their actual biological status as species or subspecies. We will address the current status of their taxonomy and its supporting evidence below, after consideration of the available genetic data.

METHODS

Genetic data. Butterflies were netted haphazardly from the locations shown in Fig. 1 (detailed in Table 1), transported alive to the laboratory on ice in individual glassine envelopes within reclosable plastic bags, and stored until analysis at −80°C. Populations were classified to species following the range maps in Opler (1992), Opler and Krizek (1984), and Scott (1986a). We used standard electrophoretic protocols from our laboratory (described in Porter and Matoon 1989) and stained the following enzyme loci: aldolase (ALDO), adenylate kinase (AK), fumarase (FUM), glutamic-oxaloacetic transaminase (2 loci: GOT-1, GOT-

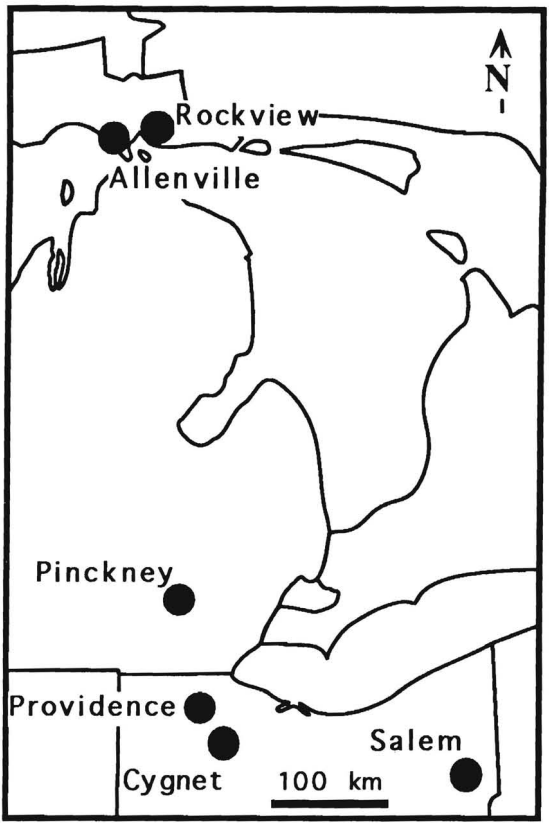

FIG. 1. Sampling localities used in this study. Opler and Krizek (1984) estimates that *tharos* extends to the top of lower Michigan on the eastern half of the state, whereas *cocyta* (nee *selenis*) extends throughout northern Michigan and to the Michigan-Indiana line in western lower Michigan; the two taxa would overlap little in Michigan. Following Opler, the Allenville and Rockview sites are *P. cocyta*, and the remainder are *P. tharos*. However, Scott (1986a) estimates *tharos* to extend north across the middle of lower Michigan and adjacent Ontario, and *cocyta* to extend south throughout southern Ontario and along the Michigan-Ohio line. The Pinkney sample thus falls within his range of overlap.

2), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), α-glycerophosphate dehydrogenase (aGPDH), isocitrate dehydrogenase (2 loci: IDH-1, IDH-2), malate dehydrogenase (2 loci: MDH-1, MDH-2), malic enzyme (2 loci: ME-1, ME-2), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucose isomerase (PGI; = PHI in Vawter & Brussard 1975), and phosphoglucomutase (PGM). Ambiguous alleles were rerun side-by-side on the same gels to confirm scoring. All statistical analyses of the allozyme data were done using a computer program written by AHP.

TABLE 1. Sample localities, capture dates, sample sizes (n), and taxonomic affinities.

#	Population	Date	n	Taxon
1	Cygnet, Wood Co., Ohio	5 Sep 1995	26	*P. tharos*
2	Providence Metropark, Lucas Co., Ohio	30 Jul 1995	31	*P. tharos*
3	Salem, Columbiana Co., Ohio	4 Jun 1995	19	*P. tharos*
4	Pinckney, Washtenaw Co., Michigan	15 Jun 1995	20	*P. tharos*
5	5 mi S Allenville, Mackinac Co., Michigan	29 Jul 1995	25	*P. cocyta*
6	10 mi WSW Rockview, Mackinac Co., Michigan	30 Jul 1995	7	*P. cocyta*

Measurement of genetic isolation. We used hierarchical *F* statistics (Wright 1969, 1978, Porter 1990, Porter & Geiger 1995) to describe allozyme variation among populations. Unlike standard genetic distance/identity measures, *F* statistics describe genetic diversity among populations in a way that can be used to estimate underlying rates of genetic exchange (Slatkin 1987, Cockerham & Weir 1993), such that we can legitimately infer process from pattern. At larger geographic scales, they may be used to measure rates of introgression and its complement, genetic isolation, and are valuable tools for testing hypotheses about species status (Porter 1990). As many readers of this journal may not be familiar with this approach, we provide a more detailed description in the Appendix. Further introduction to the population-genetic principles underlying this method may be found in introductory texts (e.g., Hartl & Clark 1997).

It is possible to estimate the rate of gene exchange among subpopulations using $M \approx (1/F_{ST} - 1)/4$ (Slatkin 1987, Cockerham & Weir 1993), where M is the effective number of individuals (or gene copies) exchanged among subpopulations per generation, and F_{ST} describes differentiation among subpopulations. This method extends across hierarchical scales, such that gene exchange may be estimated among demes within subpopulations, or among taxonomic groups (Porter 1990). Here, we use $M \approx (1/F_{GT} - 1)/4$ to estimate the degree of gene exchange between *P. tharos* and *P. cocyta*. It is a basic result of population genetics that a gene exchange rate among populations of about 1 individual (or 2 gene copies) per generation is enough to produce important homogenization of gene frequencies, regardless of population size (Wright 1931, 1969, 1978). For neutral loci, differentiation among subpopulations (F_{ST}) or taxa (F_{GT}) is produced by genetic drift and is counteracted by gene flow. Balancing selection on allozymes probably plays a negligible role in subpopulation differentiation unless the subpopulations are quite large (Porter 1990, Porter & Geiger 1995). Caution must be used in interpreting these estimates because the estimation of gene flow requires that genetic differentiation be near the equilibrium level produced by the opposing effects of gene flow and drift. If it is not, the direction of

the bias depends on whether contact among subpopulations is of primary or secondary origin. When it is out of equilibrium because of the interruption of gene flow, gene flow (M) will be overestimated, and this is especially so in large populations or over geographic regions, because genetic drift will act more slowly therein (Porter & Geiger 1995). In cases where taxon-wide differentiation has not yet equilibrated, hybrid zone analyses may then provide information of value in determining reasons for the deviation from equilibrium (Porter and Geiger 1995). However, if gene flow is estimated to be low (say, $M < 0.1$), then this value has been attained in the face of historical biases, and genetic isolation becomes a plausible explanation (Porter 1990).

Morphological data. Antennal clubs were scored for color (orange or black) and geographic variation assessed using a contingency table test. We scored the color of the ventral medial background, which forms a net-like pattern among the underside pattern elements; this reportedly tends to be black in *P. tharos* and orange in *P. cocyta*. We also scored the color of the ventral hindwing's postmedial crescent mark, which reportedly tends to be white in *P. tharos* and creamy or obscured with brown in *P. cocyta*. Correlations were calculated to confirm the degree of association among these putatively diagnostic traits; strong correlations would confirm the impressions of previous workers and tend to favor a genetic isolation interpretation. More detailed measurements of the complex patterns of wing coloration require breeding in controlled environments for interpretation of the polyphenism involved, and are beyond the scope of this paper. Wings, antennae and genitalia were saved as vouchers and are deposited in the insect collection of the Entomology Department at the University of Massachusetts at Amherst.

RESULTS

Allozyme variation within populations. Allele frequencies for the polymorphic loci are given in Table 2. ALDO, FUM and GAPDH showed no polymorphism and are omitted from the table. PGM, discussed below, was not scorable and omitted from further analyses. 6-PGD showed useful polymorphism but did not resolve in over half the individuals, and did not resolve in any of the seven individuals in population 6. For statistical reasons this locus was omitted from among-population comparisons.

Standard genetic variability scores are given in Table 3. We found high levels of genetic variability at several loci. PGI yielded 16 identifiable alleles with up to 9 alleles in a single population, and both GOT-1 and ME-1 showed 7 distinct alleles. These are higher levels of variability than reported by Vawter and Brussard (1975), though their sample sizes were comparable.

TABLE 2. Allele frequencies (standard errors) of polymorphic loci for all populations. Sample sizes for individual loci are given in brackets if they differ from those in Table 1.

	Cygnet, Ohio	Providence, Ohio	Salem, Ohio	Pinckney, Michigan	Allenville, Michigan	Rockview, Michigan
AK-1						
A		0.016 (0.016)	0.026 (0.026)		0.020 (0.020)	
B	0.981 (0.019)	0.984 (0.016)	0.974 (0.026)	0.975 (0.025)	0.960 (0.028)	1.000 (0.000)
C	0.019 (0.019)			0.025 (0.025)	0.020 (0.020)	
GOT-1		[30]			[24]	
A				0.025 (0.025)		
B		0.083 (0.036)	0.026 (0.026)		0.021 (0.021)	
C		0.033 (0.023)		0.050 (0.034)	0.542 (0.072)	0.500 (0.134)
D	0.077 (0.037)				0.021 (0.021)	
E	0.904 (0.041)	0.850 (0.046)	0.947 (0.036)	0.925 (0.042)	0.417 (0.071)	0.500 (0.134)
G	0.019 (0.019)	0.017 (0.017)				
H		0.017 (0.017)	0.026 (0.026)			
GOT-2						
A			0.026 (0.026)	0.025 (0.025)		
C	0.020 (0.020)	0.048 (0.027)	0.026 (0.026)	0.050 (0.034)	0.080 (0.038)	0.143 (0.094)
D	0.920 (0.038)	0.903 (0.038)	0.895 (0.050)	0.825 (0.060)	0.880 (0.046)	0.714 (0.121)
E					0.020 (0.020)	0.143 (0.094)
F	0.060 (0.034)	0.048 (0.027)	0.053 (0.036)	0.100 (0.047)	0.020 (0.020)	

TABLE 2. Continued.

	Cygnet, Ohio	Providence, Ohio	Salem, Ohio	Pinckney, Michigan	Allenville, Michigan	Rockview, Michigan
aGPDH						
A			0.026 (0.026)			
B	1.000 (0.000)	1.000 (0.000)	0.947 (0.036)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
C			0.026 (0.026)			
IDH-1		[29]				
A					0.020 (0.020)	
B		0.017 (0.017)			0.040 (0.028)	
C		0.017 (0.017)				
D	1.000 (0.000)	0.966 (0.024)	0.974 (0.026)	0.950 (0.034)	0.920 (0.038)	1.000 (0.000)
E			0.026 (0.026)	0.050 (0.034)	0.020 (0.020)	
IDH-2	[15]	[18]	[12]	[17]		
A		0.028 (0.027)	0.083 (0.056)	0.059 (0.040)	0.020 (0.020)	0.143 (0.094)
B	0.900 (0.055)	0.889 (0.052)	0.833 (0.076)	0.941 (0.040)	0.960 (0.028)	0.857 (0.094)
C	0.067 (0.046)	0.083 (0.046)				
D	0.033 (0.033)		0.083 (0.056)		0.020 (0.020)	
MDH-1				[19]	[24]	
A				0.026 (0.026)	0.021 (0.021)	
B	0.096 (0.041)	0.194 (0.050)	0.158 (0.059)	0.474 (0.081)	0.958 (0.029)	0.929 (0.069)
C	0.865 (0.047)	0.806 (0.050)	0.842 (0.059)	0.500 (0.081)	0.021 (0.021)	0.071 (0.069)

TABLE 2. Continued.

	Cygnet, Ohio	Providence, Ohio	Salem, Ohio	Pinckney, Michigan	Allenville, Michigan	Rockview, Michigan
D	0.019 (0.019)					
E	0.019 (0.019)					
MDH-2						
A	1.000 (0.000)	0.984 (0.016)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)	1.000 (0.000)
B		0.016 (0.016)				
ME-1						
B	0.212 (0.057)	0.419 (0.063)	0.289 (0.074)	0.325 (0.074)	0.440 (0.070)	0.643 (0.128)
C	0.019 (0.019)	0.016 (0.016)	0.053 (0.036)			
D	0.212 (0.057)	0.194 (0.050)	0.211 (0.066)	0.225 (0.066)	0.280 (0.063)	0.143 (0.094)
F	0.288 (0.063)	0.258 (0.056)	0.132 (0.055)	0.200 (0.063)	0.140 (0.049)	0.143 (0.094)
G	0.250 (0.060)	0.081 (0.035)	0.211 (0.066)	0.125 (0.052)	0.140 (0.049)	0.071 (0.069)
H		0.032 (0.022)	0.105 (0.050)	0.100 (0.047)		
I	0.019 (0.019)			0.025 (0.025)		
ME-2	[10]	[21]	[16]	[15]	[20]	
A			0.156 (0.064)	0.333 (0.086)		
B	1.000 (0.000)	1.000 (0.000)	0.812 (0.069)	0.633 (0.088)	1.000 (0.000)	1.000 (0.000)
C			0.031 (0.031)	0.033 (0.033)		
6-PGD	[16]	[15]	[4]	[10]	[9]	[0]
A	0.938 (0.043)	1.000 (0.000)	0.750 (0.153)	0.900 (0.067)	0.889 (0.074)	
B	0.062 (0.043)					

TABLE 2. Continued.

	Cygnet, Ohio	Providence, Ohio	Salem, Ohio	Pinckney, Michigan	Allenville, Michigan	Rockview, Michigan
C			0.250 (0.153)		0.111 (0.074)	
D				0.100 (0.067)		
PGI					[24]	
A		0.016 (0.016)				
B	0.019 (0.019)	0.016 (0.016)	0.026 (0.026)			
C	0.231 (0.058)	0.194 (0.050)	0.211 (0.066)	0.200 (0.063)	0.021 (0.021)	
D				0.050 (0.034)	0.021 (0.021)	
E	0.442 (0.069)	0.274 (0.057)	0.342 (0.077)	0.400 (0.077)	0.562 (0.072)	0.429 (0.132)
F	0.058 (0.032)					
G	0.019 (0.019)	0.081 (0.035)	0.053 (0.036)	0.025 (0.025)	0.021 (0.021)	0.071 (0.069)
H	0.154 (0.050)	0.371 (0.061)	0.237 (0.069)	0.250 (0.068)	0.208 (0.059)	0.500 (0.134)
I	0.019 (0.019)	0.032 (0.022)			0.021 (0.021)	
J			0.026 (0.026)		0.062 (0.035)	
K			0.079 (0.044)			
L	0.038 (0.027)			0.025 (0.025)	0.062 (0.035)	
M	0.019 (0.019)					
P			0.026 (0.026)	0.050 (0.034)		
Q		0.016 (0.016)				
W					0.021 (0.021)	

TABLE 3. Genetic variability scores for all populations, averaged over loci. A = mean alleles/locus; %P = percent of loci polymorphic; H_{obs} = proportion of individuals observed to be heterozygous; H_{exp} = proportion of heterozygous individuals expected from allele frequencies, assuming Hardy-Weinberg genotypic proportions. Standard errors, from jackknife estimates, given in parentheses. 6-PGD variability is not included in the Rockview scores because it did not resolve in those individuals.

#	Population	A	%P	H_{obs}	H_{exp}
1	Cygnet, Ohio	2.85 (0.20)	61.5 (4.2)	0.147 (0.021)	0.184 (0.022)
2	Providence, Ohio	2.92 (0.18)	69.2 (4.0)	0.189 (0.022)	0.196 (0.022)
3	Salem, Ohio	3.08 (0.16)	84.6 (3.1)	0.239 (0.028)	0.255 (0.022)
4	Pinckney, Michigan	2.85 (0.16)	76.9 (3.7)	0.268 (0.026)	0.262 (0.023)
5	Allenville, Michigan	3.08 (0.18)	69.2 (4.0)	0.163 (0.018)	0.204 (0.021)
6	Rockview, Michigan	1.83 (0.09)	50.0 (4.7)	0.190 (0.022)	0.202 (0.022)

A possibility exists that *P. tharos* and *P. cocyta* are truly genetically isolated and that we sampled both taxa in some localities, in which case diagnostic allozyme markers at different loci would be correlated within individuals. As a weak test, we therefore estimated linkage disequilibrium (a correlation coefficient) between GOT-1 and MDH-1, the only partially diagnostic markers we found (see below), in all populations. None differed significantly from zero, giving no evidence that two independent species are inadvertently mingled within our population samples. However, given that the allele frequencies at GOT-1 do not differ profoundly between taxa, this test is only likely to be strong enough to detect samples where the taxa are present in roughly equal numbers.

Variation among populations and genetic isolation. There were no fixed differences among populations at any loci, but two loci showed marked geographic differentiation in frequency. Populations 5 and 6 from Mackinac County, Michigan, in the Upper Peninsula, differed from the remaining populations especially at MDH-1 and GOT-1, with the Pinckney, Michigan, population showing intermediate frequencies at MDH-1. It is perhaps easiest to visualize these patterns using phenograms constructed from genetic distance/identity measures. The overall divergence as estimated from Nei's (1978) unbiased genetic distance and identity measures is shown in Fig. 2a, where the Mackinac Co. Michigan, populations (*P. cocyta*) appear as a distinct group. This pattern is confirmed with a second analysis using Rogers' (1972) genetic distance, and is displayed (Fig. 2b) in a tree built using the distance-Wagner algorithm described in Ferris (1972).

Results of the hierarchical F-statistical analysis are shown in Table 4. F_{SG} describes the differentiation among gene frequencies of the populations in Table 2 from the average frequencies of their respective groups, *P. tharos* and *P. cocyta*. The differentiation is low, and may be explained by an average gene exchange rate of $\hat{M}$ = 7.6 (95% c.i. 5.2–13) individu-

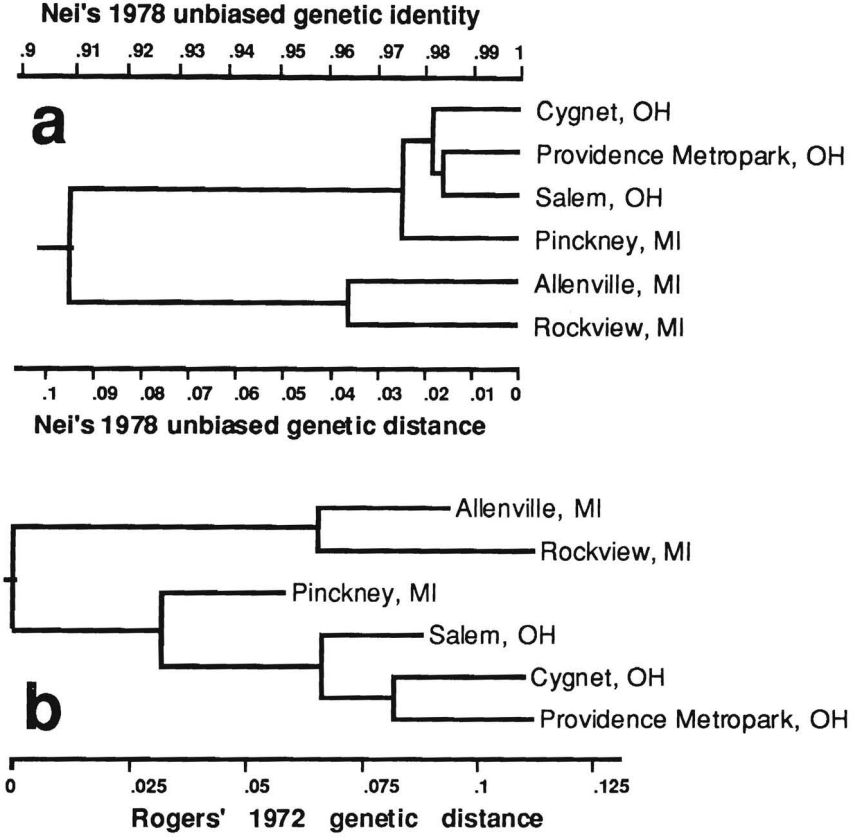

FIG. 2. Phenograms describing genetic differentiation among populations. a, UPGMA (Sokal & Sneath 1963) phenogram based on Nei's (1978) unbiased distance and identity measures. b, Distance-Wagner tree (Ferris 1972) based on Rogers' (1972) genetic distance. *P. cocyta* populations from Mackinac Co., Michigan fall into a distinct group in both measures.

als per generation within these two taxa. F_{GT} describes differentiation of gene frequencies between those population groups designated as *P. tharos* and *P. cocyta*. This differentiation is higher, but still permits a gene exchange rate between these taxa of $\hat{M} = 0.88$ (0.54–1.7) individuals per generation. This value suggests incomplete genetic isolation, particularly in comparison to the high within-taxon values (Porter 1990, Porter & Geiger 1995).

Morphological traits. We found no support for the hypotheses that the antennal club colors, ventral hindwing ground color, or the color of the underside crescent were useful for taxonomic diagnosis of *P. tharos* and *P. cocyta*. There were significant differences among popula-

TABLE 4. *F* statistics describing genetic divergence at different hierarchical levels, and estimates of the rates of genetic exchange ($\hat{M}$) at these levels. Upper and lower bounds of $\hat{M}$ represent 95% confidence intervals derived from jackknife variance estimates on the *F* statistics, taken over the 11 polymorphic loci (omitting 6-PGD).

Hierarchical level		Value	(SE)	Lower bound	$\hat{M}$	Upper bound
F_{GT}	between *tharos* & *cocyta*	0.221	0.014	0.54	0.88	1.7
F_{ST}	among all populations	0.246	0.015	0.48	0.77	1.4
F_{SG}	among populations within *tharos* & *cocyta*	0.0319	0.0021	5.2	7.6	13
F_{IT}	within individuals	0.289	0.015			
F_{IG}	within individuals, within *tharos* & *cocyta*	0.0873	0.0038			
F_{IS}	within individuals, within populations	0.0572	0.0043			

tions in the frequencies of black vs. orange antennal color morphs in both males and females (Table 5), but these were not associated with the taxonomic groupings. This supports Scott's (1986a, 1994) observation that the antennal color is ambiguous in the northern part of the range of *P. tharos*. We found what appeared to be continuous variation in the color of the ventral crescent, and also in the degree of black scaling mixed into the ventral ground color. When all populations were pooled, we found an expected correlation between sex and the color of the ventral crescent ($r = -0.53$, $p < 0.001$), but no other significant associations among sex, antennal club color, crescent color, and ventral ground color. When we broke this down by population, we found additional significant ($p < 0.05$) correlations between sex and antennal color in Pinckney and Salem (cf. Table 5), crescent and antennal color at Salem (probably acting through the previous correlation), and antennal and ground color at Pinckney. Within females, we also found significant positive correlations between ventral ground color and crescent color in Pinckney and Al-

TABLE 5. Antennal club color frequencies for each population. For males, significant differences are due to the higher frequency of black-clubbed individuals at Salem, Ohio. For females, significance is due to the higher frequencies of black morphs in Pinckney and Rockview. These do not correlate to the taxonomic affinities.

	P. tharos				*P. cocyta*			
Color	Cygnet, Ohio	Providence, Ohio	Salem, Ohio	Pinckney, Michigan	Allenville, Michigan	Rockview, Michigan	G	p <
♂♂								
orange	19	17	4	12	11	2	−49.03	0.001
black	3	4	8	0	1	0		
♀♀								
orange	4	8	6	4	10	3	−80.47	0.001
black	0	2	1	4	3	2		

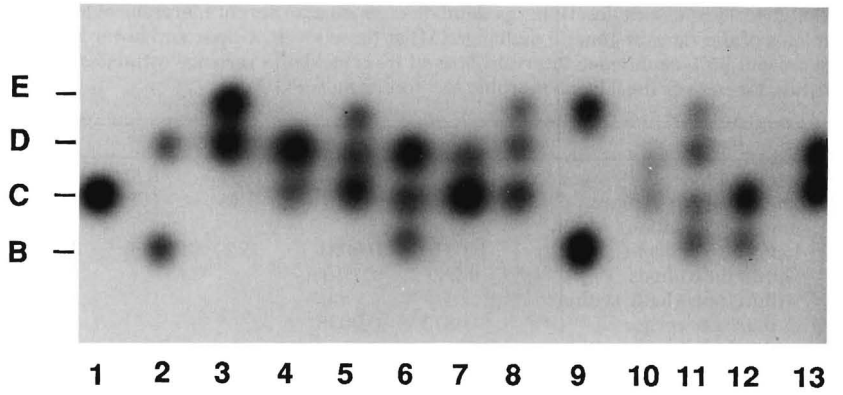

FIG. 3. Representative zymogram showing duplication of the PGM locus. Individuals are numbered and electromorphs are lettered. Alleles of the two loci overlap and cannot be assigned to the separate loci, but the genotypes can be estimated from the relative banding intensity, with relatively darker bands indicating that more than one allele has migrated to that location. The combined 2-locus genotypes seen here are: 1) CCCC, 2) BBDD; 3) DDEE; 4) CDDD; 5) CCDE; 6) BCDD; 7) CCCD; 8) CCDE; 9) BBEE; 10) CCDD; 11) BCDE; 12) BCCC; 13) CCDD. 1–5: Salem, Ohio; 6–10: Pinckney, Michigan; 11–13: Providence, Ohio, all *P. tharos*; similar patterns were found in the *P. cocyta* populations.

lenville, suggesting general ventral melanization effects there. These patterns varied geographically and were not sufficiently strong to form a basis for taxonomic diagnosis. We were not able to confirm Oliver's (1980) qualitative descriptions of differences in dorsal wing pattern traits between *P. tharos* and *P. cocyta*.

The PGM locus. PGM is a monomeric enzyme (comprised of a single protein unit), so heterozygotes are expected to show a pair of bands and homozygotes show a single band. In both *P. tharos* and *P. cocyta*, individuals show up to 4 bands (Fig. 3), suggesting that gene duplication may have occurred. Individuals with 2–3 bands show variation in the relative intensity of those bands, and all individuals with 3 bands show one or another of their bands of greater intensity, in a manner consistent with the interpretation that alleles of two PGM loci migrate to overlapping locations on the gel. These banding patterns are well-resolved, repeatable, and not consistent with simple patterns of secondary banding caused by degradation, sometimes seen in other loci. However, without breeding studies, it is not possible to assign electromorphs to the two PGM loci. Vawter and Brussard (1975) reported only a single PGM locus with typical patterns of variability, and we cannot explain the discrepancy. If verified by breeding and molecular methods, a duplication of PGM would make a good derived trait for phylogenetic studies, as was recognized for the duplicated PGI locus in *Clarkia* (Onagraceae) (Gottlieb & Weeden 1979).

DISCUSSION

One species or two? The allozyme analyses presented here (Table 4) support the interpretation that *P. tharos* and *P. cocyta* are incompletely isolated from one another genetically. Although the amount of genetic differentiation observed between the two taxa is higher than that observed among populations within them, biologically important genetic exchange rates of approximately one genome per generation (Wright 1931, 1969, 1978, Slatkin 1987) across the taxonomic boundary are still consistent with the patterns we observed.

This interpretation is in full agreement with Oliver's breeding data. He found that F_1 hybrid and backcross broods had high fertility and survivorship rates, with developmental incompatibilities accounting for only a 15% reduction in survivorship, a negligible barrier to gene exchange. Scott (1986b) released Rocky Mountain *P. cocyta* females in front of *P. tharos* males and thereby readily obtained natural matings and fertile offspring, indicating that there is presently no evidence for mating barriers between these taxa. All the currently available evidence indicates that when these taxa do come into contact, avenues exist for considerable introgression between them. From this perspective, these taxa might best be considered taxonomically as subspecies, whereupon the name *tharos* is the oldest available.

How then might the putatively diagnostic traits be maintained in the face of homogenizing gene flow? If there are plausible explanations for the maintenance of the differences we observe between taxa in the *Phyciodes tharos* group, then it would be premature to reach the conclusion that genetic isolation is responsible. This is especially so because the putatively diagnostic traits are apparently not correlated with one another (Tables 4, 5).

Within a species, there is a simple adaptive explanation available for the primary diagnostic-trait difference that Oliver (1980, 1983) identified between *tharos* and *cocyta*, namely the association between body size, development time, and the number of annual generations along the geographic line of transition between one generation and two. Roff (1983) developed a general model of these transitional regions in voltinism. He demonstrated that just north of such a line, where a single generation is favored, individuals may increase their fitness by prolonging development. This permits them to reach larger adult body sizes, with concomitant increases in fecundity, without selective pressure to reach the appropriate diapause stage before the season ends. This life-history strategy begins to backfire further northward in the range, where the season ends sooner and selection favors more rapid development in order to simply reach the diapause stage. Just south of the transition line,

two generations are favored, but only for those individuals able to develop fast enough that the second generation is able to diapause. This requires individuals to mature at smaller body sizes, as the fitness advantage of having an additional generation outweighs the costs on individual fecundity of smaller body size. Further south, the time window is longer, and selection favors larger body sizes even with two generations. As these life-history traits are closely tied to fitness, the selection acting upon them is likely to be strong enough to overcome the homogenizing gene flow rates of $M \approx 1$ individual per generation that we observed across the taxonomic boundary. Burns (1985) suggested a similar explanation for the correlation between body size and voltinism in the hesperiid *Wallengrenia egeremet* (Scudder).

Patterns of variation in the remaining traits have not yet been documented well enough quantitatively to be able to determine their contribution to genetic isolation, and the qualitative impressions made "by eye" of population averages, used to date in the taxonomic research of this group, are inadequate to this task. The wing patterns are highly variable within populations and show seasonal adaptations. Given the developmental complexity of pattern elements on the wing surface (Nijhout 1991), adequate statistical description of geographic variation in wing pattern will be a major undertaking, and a quantitative understanding of its genetic basis as it relates to genetic isolation may be formidable. But until the patterns of variation are adequately described, we cannot hope to distinguish among their possible causes. The genitalic differences reported by Scott (1986a, 1994) are subtle, and such differentiation is not necessarily indicative of reproductive isolation (Shapiro & Porter 1989, Porter & Shapiro 1990), a point Scott (1994) acknowledges. They may be maintained, for example, by sexual selection during copulation (Eberhard 1985) or even as neutral traits in an isolation-by-distance population structure (Endler 1977). It is difficult to imagine the larval color patterns contributing in a significant way to genetic isolation. The known level of geographic variation in this suite of diagnostic traits remains consistent with a pattern of partial genetic isolation between *P. tharos* and *P. cocyta*.

Does the differentiation observed to date indicate secondary contact between taxa evolved in allopatry, or simple geographic differentiation without separation? One can only speculate. The geographic location of the contact area in eastern North America was glaciated 18,000 years ago, and is likely to have achieved its present ecosystem (barring very recent human population growth) only in the last 8000 years. Given that the present contact area runs west to east, and glacial recession proceeded from south to north, the biogeography of range changes involving any hypothesized allopatry would have had to be complex. A com-

plex history in this region can be found in genetic patterns from the *Papilio machaon* (L.) group taxa (Sperling 1993), but suitable data are not available to address this issue in *Phyciodes*.

In summary, there is yet no *prima facie* evidence of separate species status for *P. tharos* and *P. cocyta* and it is at best premature to separate them. Field collections yield intermediate specimens, and laboratory studies of the life cycles indicate that the two taxa respond similarly to spring-time conditions that determine the timing of the first adult generation (Oliver 1980), suggesting that the taxa are not temporally isolated in sympatry. For the maintenance of the life-history differences, there is a reasonable, adaptive explanation available that does not require genetic isolation. Several putatively diagnostic morphological differences were not well supported in our correlational analyses. Remaining differences proposed by various workers, and untested by us, require quantitative analysis and more certain evidence of genetic isolation as their cause. The genetic data, for which the best methods are currently available to link patterns of genetic variation to gene flow barriers, do suggest that a weak barrier exists. However, this barrier is analogous more to a sieve than a wall, in that it is too weak to inhibit for more than a few generations neutral and adaptive genetic exchange between the taxa, even when the confidence limits are considered. The genetic analysis too is preliminary: if genetic isolation has in fact evolved only very recently, it remains possible for the regional patterns of differentiation we observed between the taxa to have had insufficient time to drift apart (Porter & Geiger 1995). Therefore, studies of the contact area on a finer scale are needed to test and resolve the results we present here.

The *Phyciodes tharos/cocyta* system is a prime candidate for study using hybrid zone theory (Barton & Gale 1993). The taxa are parapatrically distributed, with a defined region of contact and apparent hybridization in the center. There are ecological conditions capable of producing adaptive differences in the regulation of diapause on either side, and available allozyme markers with which to document cline shape. This body of theory is useful for interpreting the relationships between pattern and underlying evolutionary processes, regardless of whether the taxa are in secondary contact or have reached their present level of differentiation without separation (Harrison 1993), and regardless of the width of the contact region (Barton & Gale 1993). These models permit the estimation of the strength of natural selection acting both in the zone itself and out in the tails of introgression, and permit the estimation of the rate of introgression (Barton & Gale 1993, Porter et al. 1997b). If such introgression resolves to be negligible, this would comprise *prima facie* evidence for genetic isolation and it would be appropriate to reassess the taxonomic status of this group.

Selection, gene flow and allozyme variation. There have been significant advances in our understanding of the causes of geographic patterns of allozyme diversity since the study by Vawter and Brussard (1975). There is no need to invoke strong stabilizing selection for the allozyme similarities in the *P. tharos* system, as gene flow rates of several individuals entering a population each generation are sufficient to maintain the observed levels of variation. Nevertheless, very weak selection may account for high similarity over very large areas, as the effective population sizes are so much higher on regional spatial scales that very weak stabilizing selection (on the order of the mutation rate) can maintain range-wide similarity (Porter & Geiger 1995). Such subtle processes may be unmeasurable in natural populations, where the stochastic effects of genetic drift may overcome the weak deterministic forces of selection at the local spatial and temporal scales to which field biologists, for logistic reasons, are confined.

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APPENDIX

F statistics summarize the extent to which populations deviate from Hardy-Weinberg genotypic proportions. Populations in Hardy-Weinberg equilibrium proportions have $F = 0$. Populations with excesses of homozygotes have $F > 0$ to a maximum of $F = 1$; those with heterozygote excesses have $F < 0$ to a minimum of $F = -1$. A population may be hierarchically organized as a group of subpopulations (the terms 'population' and 'subpopulation' are simply statistical constructs which may or may not be identical to populations in the biological sense), whereupon *F* in the total population, denoted F_{IT}, may be hierarchically partitioned to describe averages of deviations from Hardy-Weinberg expectations on different geographic scales. The partitioning is $(1-F_{IT}) = (1-F_{IS})\,(1-F_{ST})$ (Wright 1951, 1978), where F_{IS} describes the average of deviations from Hardy-Weinberg proportions within the subpopulations, and F_{ST} describes the component of overall deviation produced by gene frequency differences among subpopulations. Other hierarchical levels are often inserted, as we describe below. On local scales, within subpopulations, deviations from Hardy-Weinberg expectations arise from nonrandom mating, and F_{IS} may be positive or negative. At larger scales, only positive values (aside from sampling variation) may arise in F_{ST}, when the subpopulations have different allele frequencies. This is easiest to imagine in the extreme case where subpopulations are fixed for different alleles, yielding a complete deficiency of heterozygotes in the total population. As such, F_{ST} is a common statistic describing average differentiation among subpopulations.

F statistics have historically been derived mathematically from three additional perspectives: as the degree to which alleles identical-by-descent are distributed within and among individuals (*f* of Malécot 1969) and populations (*G* of Nei 1973); by path analysis as correlations between alleles in uniting gametes (i.e., within diploid individuals) within and among subpopulations (*F* of Wright 1951, 1978); and from a nested analysis of variance model describing the pattern in which total allelic variation is partitioned among subpopulations, among individuals within subpopulations, and between pairs of alleles within (diploid) individuals (Cockerham 1969, 1973, Weir & Cockerham 1984). Although the derivations yield the same analytical result and their various interpretations are biologically equivalent, the third approach lends itself most readily to statistical treatment of data because degrees of freedom can easily be incorporated at several sampling levels (Weir & Cockerham 1984, Weir 1990).

Because of the hierarchical structure of *F*-statistics, additional levels may be readily included in analyses, representing, for example, subdivisions of large subpopulations into

demes (Wright 1978) or grouping of subpopulations into intraspecific geographical or taxonomic units (Porter 1990, Porter & Geiger 1995, Porter et al. 1997a). In this study, we investigate differentiation at three hierarchical levels: between taxonomic units (F_{CT}), namely *P. tharos* and *P. cocyta*, among subpopulations within taxonomic units (F_{SC}), and among individuals within subpopulations (F_{IS}). The partitioning is thus $(1-F_{IT}) = (1-F_{IS})(1-F_{SC})(1-F_{CT})$. These hierarchical levels may be collapsed to yield $(1-F_{IT}) = (1-F_{IS})(1-F_{ST})$ and $(1-F_{IT}) = (1-F_{IG})(1-F_{GT})$ as above, and we also draw inferences from F_{ST}. We used Weir and Cockerham's (1984) statistical estimators for *F*-statistics, derived from the unbiased hierarchical variance components a, b_1, b_2, and c, as defined in their paper. These estimators are:

$F_{IS} = 1 - (c / (b_1 + c))$
$F_{IG} = \hat{f} = 1 - (c / (b_1 + b_2 + c))$
$F_{IT} = \hat{F} = 1 - (c / (a + b_1 + b_2 + c))$
$F_{SG} = 1 - ((b_1 + c) / (b_1 + b_2 + c))$
$F_{ST} = \theta_1 = 1 - ((a + b_2) / (a + b_1 + b_2 + c))$
$F_{GT} = \theta_2 = 1 - (a / (a + b_1 + b_2 + c))$

where θ_1, θ_2, $\hat{F}$, and $\hat{f}$ follows their notation; the "hat" indicates that the quantity is being estimated from data. Single-locus estimates were combined over loci and alleles using a weighted average, and error estimates were obtained by the "jackknife" resampling method (Weir & Cockerham 1984).

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