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Does size matter? Comparative population genetics of two butterflies with different wingspans

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Abstract The dispersal ability of a species is central to its biology, affecting other processes like local adaptation, population and community dynamics, and genetic structure. Among the intrinsic, species-specific factors that affect dispersal ability in butterflies, wingspan was recently shown to explain a high amount of variance in dispersal ability. In this study, a comparative approach was adopted to test whether a difference in wingspan translates into a difference in population genetic structure. Two closely related butterfly species from subfamily Satyrinae, family Nymphalidae, which are similar with respect to all traits that affect dispersal ability except for wingspan, were studied. *Melanitis leda* (wingspan 60–80 mm) and *Ypthima baldus* (wingspan 30–40 mm) were collected from the same areas along the Western Ghats of southern India. Amplified fragment length polymorphisms were used to test whether the species with a higher wingspan (*M. leda*) exhibited a more homogenous population genetic structure, as compared to a species with a shorter wingspan (*Y. baldus*). In all analyses, *Y. baldus* exhibited greater degree of population genetic structuring. This study is one of the few adopting a comparative approach to establish the relationship between traits that affect dispersal ability and population genetic structure.

Keywords Amplified fragment length polymorphisms · Butterflies · Dispersal ability · Population genetic structure · Western Ghats · Wingspan

Introduction

Dispersal is a central process in the ecology of a species, affecting a variety of other processes, from local adaptation, population and community dynamics, to genetic structure (Doligez and Part 2008). From a contemporary perspective, dispersal ability can affect the survival of a species in a fragmented landscape: species with greater dispersal ability will be better able to move between patches of suitable habitat (Baguette et al. 2003; Doligez and Part 2008). The metapopulation concept (Hanski and Gaggiotti 2004) provides a framework for studying fragmented populations at a landscape level, where the survival of a species in the region is determined by the colonization and extinction of subpopulations. Increased dispersal leads to higher levels of gene flow, defined as "the successful movement of alleles (via permanent migrants, fertilized gametes and established propagules) between and within populations" (Lowe et al. 2004).

Gene flow, or lack thereof, gives rise to patterns in the spatial and temporal distribution of genetic variation, which can be studied as a phylogeographic pattern at a broad scale, and as population genetic structure at local scales (Bohonak 1999). There is thus a causal relationship between dispersal, gene flow, and genetic structure—intuitively, higher dispersal ability would lead to higher gene flow over a larger area, increasing homogeneity in the genetic structure across subpopulations of the species (Schmitt and Hewitt 2004). Lower dispersal ability, on the contrary, would result in increased genetic structuring and decreased genetic diversity in small isolated populations, which may have adverse

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consequences for fitness, and demography (Vandewoestijne et al. 2008).

Studies have investigated the relationship between the factors that affect dispersal ability and population genetic structure (Bohonak 1999; Govindaraju 1988; Peterson and Denno 1998). Many studies use the comparative approach while making inferences about the relationship between traits that affect dispersal ability and gene flow, to reduce confounding factors and make the hypothesis more robust (Bohonak 1999). The immense amount of natural history information available on butterflies makes them an excellent model system to test such comparative hypotheses.

A recent meta-analysis examined the main factors that influence dispersal ability in butterflies (Sekar 2012). Among the morphological (wingspan), ecological (larval host plant specificity, adult habitat specificity), and life history (number of generations per year, flight period duration) traits examined, wingspan emerged as a significant predictor of dispersal ability. While the predictive powers of regressions were low, effect sizes were high, firmly establishing a relationship between wingspan and dispersal ability. A similar correlation between wingspan and butterfly mobility was retrieved in Canadian butterflies (R. J. Burke et al. 2011). However, another meta-analysis (Stevens et al. 2010) does not retrieve a relationship between wingspan and butterfly dispersal ability.

In spite of the contention surrounding the relationship between wingspan and butterfly dispersal ability, many large-scale studies that use dispersal ability during the analysis use wingspan as a proxy (Cowley et al. 2001; Koh et al. 2004; Kotiaho et al. 2005; Kuefler et al. 2008; Quinn et al. 1997). This is because the information is easily available for most species, whereas measuring the actual dispersal behavior of individuals is more time-consuming and difficult (Hanski et al. 2002). At this juncture, testing the relationship indirectly, using molecular tools to analyze the relationship between wingspan, dispersal ability, and gene flow, can offer necessary insights.

Molecular studies on butterflies have established that species with a high dispersal ability have a more uniform genetic structure (Habel and Schmitt 2009; Takami et al. 2004). However, very few studies have adopted the comparative approach, and fewer still have used wingspan as a proxy for dispersal ability, and arrived at relationships between wingspan, dispersal ability, and genetic structure. One such study found that among three congeneric skipper species, the habitat specialist with small wingspan, *Thymelicus acteon*, had the most pronounced population genetic structure, while the larger habitat generalist *Thymelicus lineola* had a panmictic population genetic structure (Louy et al. 2007). This study could not tease apart the relative importance of habitat specificity and wingspan in causing a difference in population genetic structure. A similar study using three butterfly species in the same habitat found that the small specialist *Cupido minims* had maximum population genetic structure, while the larger generalist *Melanargia galathea* had panmictic structure (Baguette et al. 2000). This study compares three species with different ecologies, belonging to three different butterfly families. The relative importance of wingspan and species ecology in shaping population genetic structure could not be teased apart. Thus, to better understand the role of wingspan as a proxy for dispersal ability through its affect on population structure, it is important to control for all other confounding factors, and adopt a more robust comparative approach.

One such robust comparison is attempted in this study, by using two closely related species belonging to the family Nymphalidae, subfamily Satyrinae (Peña et al. 2006), to investigate whether species with a higher wingspan had a more homogenous population genetic structure. *Melanitis leda* (the Common Evening Brown) belongs to the tribe Melanitini, with a wingspan of 60–80 mm. The species is widespread, with its global range extending throughout Africa, Madagascar, the Arabian Peninsula, Southeast Asia, the Pacific Islands, and Australia (http://ftp.funet.fi). *Ypthima baldus* (the Common Five-Ring) belongs to the tribe Satyrini, with a wingspan of 30–40 mm. It is not as widespread as *M. leda*, occurring in South and Southeast Asia (http://ftp.funet.fi).

Apart from a difference in wingspan, where *M. leda* (henceforth ML) is roughly twice the size of *Y. baldus* (henceforth YB), the two species are similar in ecology, and life history traits: the larvae of both species are generic grass feeders, and the adults are not restricted to a particular habitat type. They have multiple reproductive cycles in a year, and are on the wing through most of the year (Kehimkar 2008). Thus, any difference in population genetic structure between the two species can more readily be attributed to a difference in wingspan, if they are sampled from the same areas to control for geography. The amplified fragment length polymorphisms (AFLPs) technique was used to compare the population genetic structure of the two species across the Western Ghats of southern India. The hypothesis was that ML, with its higher wingspan, would have higher dispersal ability, and thus would exhibit limited or no population genetic structure; the smaller YB, on the other hand, would exhibit a higher degree of genetic structuring. Apart from comparing population genetic patterns, a second morphological trait, wing aspect ratio, was also calculated and compared.

Materials and methods

Study species

M. leda (the Common Evening Brown), from the tribe Melanitini in subfamily Satyrinae, is widespread, with its global range extending throughout Africa, Madagascar, the

Arabian peninsula, Southeast Asia, the Pacific Islands, and Australia (http://ftp.funet.fi). It has a wingspan of 60–80 mm. *Y. baldus* (the Common Five-Ring) belongs to the tribe Satyrini in the same subfamily, with a wingspan of 30–40 mm. It is not as widespread as *M. leda*, occurring in South and Southeast Asia (http://ftp.funet.fi). The larvae of both species are generic grass feeders, and the adults are not restricted to a particular habitat type. They have multiple reproductive cycles in a year, and are on the wing through most of the year (Kehimkar 2008).

Sampling

Fieldwork was conducted in the Western Ghats of southern India, in low and mid elevation areas where both species are found (Wynter-Blyth 1957). Using opportunistic sampling and fruit bait traps, about 61 individuals of each species were collected over a straight-line distance of about 750 km (Fig. 1). After preserving two legs in ethanol for molecular work, all samples were deposited as voucher specimens at the Centre for Ecological Sciences, Bangalore, and at the Kerala Forest Research Institute.

Morphological analysis

Wing aspect ratio was calculated for 25 individuals of each species. Wing aspect ratio is an index of wing shape, defined as four times the square of the wing length divided by wing area (Chai and Srygley 1990). The forewings of individuals were cut out and digitized, and the forewing length and area were estimated in ImageJ (Abràmoff et al. 2004). In Lepidoptera, faster fliers tend to have shorter forewings with longer wing chords, and thus lower aspect ratios (Chai and Srygley 1990). The expectation was that ML, the better disperser, would probably also fly faster and have lower wing aspect ratio. A Mann-Whitney test was used to compare the wing aspect ratios of the two species.

Lab techniques

The extraction protocol was adapted from Reineke et al. (1998). The primers and procedures used during the restriction, ligation, and pre-selection steps of the AFLP protocol were the same as those used in Sekar and Karanth (2013). Primer pairs used for the selection step are listed in Table 1. The electropherograms were visualized and edited where necessary, using Peak Scanner (Applied Biosystems). Sample binning and scoring was performed in RawGeno (Arrigo et al. 2009). The final combined dataset had 547 loci for ML and 487 for YB.

In order to minimize error, the same person (SS) did all the lab work. Further precautions were taken as per Crawford et al. (2012). Positive and negative controls were used during extraction and the genotyping stage to ensure that between-run variability was not so high as to affect results. DNA was extracted from two samples taken from the same individual, for 10 % of the individuals of each species. The genotypes of both replicates were used to calculate error rate, using the Bayesian error estimation technique implemented in AFLP Score (Whitlock et al. 2008). The error rate was estimated to be about 4 %, which is considered acceptable for publication (Bonin et al. 2004).

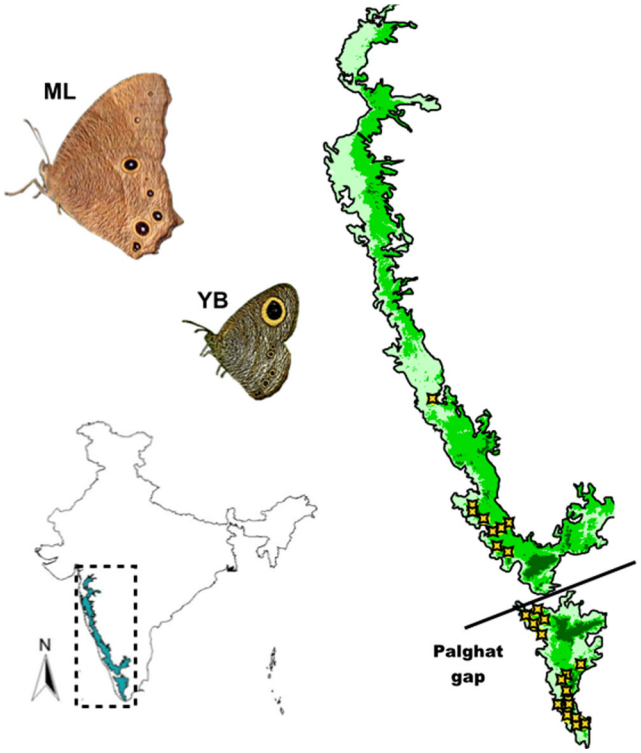

Fig. 1 Sampling locations. A map of the Western Ghats showing sampling locations for both species. *Inset*: map of India showing the location of the Western Ghats in *blue*

Molecular analyses

Two kinds of analysis were performed: (1) individual-based analyses to obtain overall patterns in the dataset—spatial autocorrelation, isolation by distance, and clustering; and (2) population-based analyses after assigning individuals into populations—genetic diversity, genetic differentiation, and assignment tests. The analyses are explained briefly in this publication; please refer to Sekar and Karanth (2013) for more details.

Overall patterns

Spatial autocorrelation was estimated in the software package GenAlex (Peakall and Smouse 2006), and both datasets were divided into five distance classes of 150 km each. The expectation was that the smaller YB would exhibit stronger spatial

Table 1 List of primer pairs used in the AFLP analysis of ML and YB

Melanitis leda			*Ypthima baldus*		
Labeled	Unlabeled	Number of loci	Labeled	Unlabeled	Number of loci
E4	M1	170	E4	M1	135
E4	M3	167	E5	M1	96
E5	M1	102	E5	M7	103
E5	M7	108	E7	M7	153
Total loci		547	Total loci		487

Fluorescent dye used for the labeled primers is mentioned in brackets. FAM: 6-FAM Fluorescein (*blue dye*) and *HEX*: hexachlorofluorescein (*green dye*)

E4* (FAM): GACTGCGTACCAATTC-CAA; E5* (HEX): GACTGCGTACCAATTC-CAT; E7* (FAM): GACTGCGTACCAATTC-CT; M1: GATGAGTCCTGAGTAAACAT; M3: GATGAGTCCTGAGTAAA-CTA; M7: GATGAGTCCTGAGTAAA-AT

autocorrelation (a higher *r*). Isolation by distance was estimated by a Mantel's test, implemented in the package ade4 (Dray and Dufour 2007) in R (The R Development Core Team 2011). To detect clusters in the datasets, a spatial Principal Components Analyses (sPCA) analysis (Jombart 2008), which does not make assumptions about linkage equilibrium or Hardy-Weinberg equilibrium, was performed. Two permutation tests were used to test for significant clustering (Jombart 2008): the Gtest (test for global structures, positive spatial autocorrelation), and Ltest (test for local structures, negative spatial autocorrelation).

Population-based analyses

In order to group individuals of both species into groups without bias, a matrix of the geographic distance between sampling coordinates was first obtained. From the matrix, the four quartiles of geographic distances were calculated. The first quartile (96 km, which was rounded off to 100 km) was used as a cutoff; all individuals separated by less than 100 km were placed in one group. The same groups could be used for both species because sampling was carried out in the same places for both. This gave rise to four groups (Fig. 2a; detailed list in Table S1, Supporting Information)—AGA (Agastyamalais hill complex and surrounding plains; n=28 for both species), ANA (Anamalais hill complex and surrounding plains; n=12), NKAR (North Karnataka; n=5), and WAC (Wayanad and Coorg; n=16). The groups obtained were used in the following analyses. For a detailed account of the analysis methods, refer to Sekar and Karanth (2013).

Genetic diversity, including heterozygosity and allele frequencies, were calculated using AFLPSurv (Vekemans et al. 2002). Genetic differentiation between the various populations of each species was estimated using band-based and allele frequency-based F_{ST} (Bonin et al. 2007; D. E. Irwin et al. 2011), Nei's D genetic distance (Lynch and Milligan 1994), also calculated using AFLPSurv. An analysis of molecular variation (AMOVA) was performed in Arlequin (Excoffier and Lischer 2010). Assignment tests were carried out in AFLPOP (Duchesne and Bernatchez 2002), using a minimum log-likelihood difference (MLD) of 1 (He et al. 2004).

Results

Wing aspect ratio

Mean wing aspect ratio of ML was 8.5, versus 9.19 for YB. The Mann-Whitney test used to compare the wing aspect ratios was significant (W=332, P=0.004185).

Molecular analyses: overall patterns

(a) *Spatial autocorrelation*

Both species exhibit significant spatial autocorrelation in the first distance class, in both one-tailed and two-tailed tests (ML: r=0.017, upper limit of permutation test, U=0.007, lower limit of permutation test, L=−0.006, P<0.0001; YB: r=0.047, U=0.009, L=−0.007, P=0.0001). r was greater in the case of YB, and both species showed significant negative spatial autocorrelation in the other four distance classes, with the magnitude of r being higher for ML (data not shown). When spatial autocorrelation patterns were compared between species, all three methods proved that there is a difference between the correlograms (r ML=0.017, 95 % bootstrap CI=0.010 to 0.020, r YB=0.047, 95 % bootstrap CI=0.035 to 0.05; T2=10.18, P=0.001; Omega=81.41, P=0.0001).

(b) *Isolation by distance*

YB exhibited significant IBD (Mantel r=0.215, P=0.0001), while ML did not (Mantel r=0.015, P=0.303).

(c) *sPCA*

After examining the screeplots of the sPCA eigenvalues, all the 3 positive eigenvalues for ML (amounting

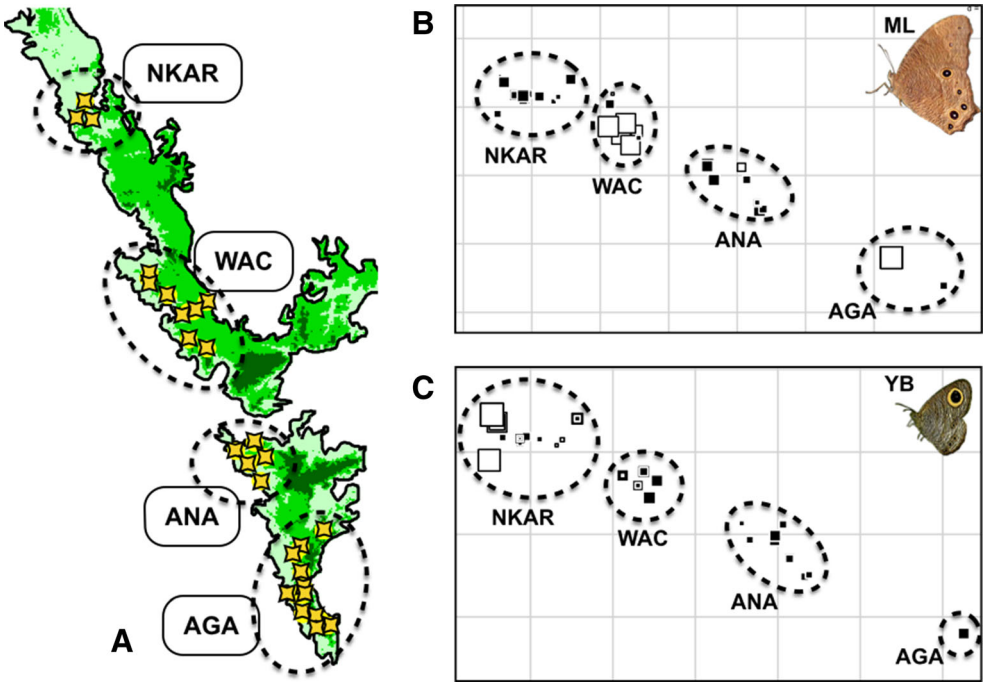

Fig. 2 **a** Map showing the groups formed when individuals were divided into populations based on sampling proximity (*AGA* Agastyamalais, *ANA* Anamalais, *WAC* Wayanad and Coorg, and *NKAR* North Karnataka). **b** and **c** Spatial multivariate analysis in sPCA. *Squares* represent first axis sPCA scores for **b** ML and **c** YB. In this figure, the color of the squares denotes positive (*black*) or negative (*white*) spatial autocorrelation, and the *size of the squares* denotes the magnitude of genetic variance. Thus, *squares of different sizes* are used to represent different absolute values: *large black squares* are well differentiated from *large white squares*, but *small squares* are less differentiated

to 7.5 % of the total variance), and the first two for YB (amounting to 10.2 % of the total variance) were retained. In both species, the Gtests were significant, while the Ltests were not (Gtest values ML: max(**t**)= 0.033, P=0.023; YB: max(**t**)=0.043, P=0.001), implying high genetic variance and higher degree of clustering than expected by chance, i.e., positive spatial autocorrelation. There was however no clear geographic clustering in either species. Furthermore, when looking at the decomposition of the first two positive sPCA scores, YB has a higher Moran's I for all the three sPCA eigenvalues that can be compared between the two species.

Molecular analyses: patterns observed between populations

(a) *Genetic diversity*

The results are summarized in Table 2. The percentage of polymorphic loci (PLP) was comparable across both species, varying from 33.5 to 59.2 % in the case of ML and from 27.7 to 56.9 % for YB. The total heterozygosity across all populations was 0.1438 for ML, and 0.1588 for YB. The expected heterozygosity (H_e) for each population did not vary much between populations for either species, ranging from 0.12 to 0.16 for ML, and from 0.12 to 0.15 for YB. A Mann-Whitney test was performed to compare PLP and H_e of the two species, and both were not significantly different.

(b) *Genetic differentiation*

The allele-based F_{ST} showed that there was significant genetic differentiation among groups in both species: for ML, overall F_{ST}=0.0323, P=0.002; for YB, overall F_{ST}=0.145, P=0.0001 (Table 3). Band-based F_{ST} (ML overall F_{ST}=0.05363, P<0.0001; YB, overall F_{ST}= 0.14534, P<0.0001) matrices followed similar trends; the between-population F_{ST} values are given in Table S2 in the Supporting Information. The Mann-Whitney tests performed to differentiate between the two species were significant for all three distance matrices—allele frequency-based, band-based, and Nei's D. In the AMOVA analysis, only about 5 % of the genetic variation was explained by variation among groups in ML; on the other hand, as much as 15 % is explained by variation among populations in YB (Table S3 in Supporting Information).

(c) *Assignment tests*

Out of 61 individuals in each dataset, 8 from the ML dataset, and 5 from the YB dataset could not be assigned

Table 2 Genetic diversity within populations of ML and YB

Population names	ML		YB	
	PLP	H_e (±SD)	PLP	H_e (±SD)
ANA	33.5	0.120 (0.005)	27.7	0.123 (0.007)
NKAR	38.6	0.145 (0.007)	32.6	0.125 (0.008)
AGA	52.5	0.122 (0.004)	53.6	0.151 (0.006)
WAC	59.2	0.169 (0.005)	56.9	0.146 (0.005)

PLP percentage of polymorphic loci, H_e expected heterozygosity (±standard deviation), *ANA* Anamalais, *NKAR* North Karnataka, *AGA* Agastyamalais, *WAC* Wayanad/Coorg complex

Table 3 Genetic differentiation matrices among populations in a) ML and b) YB datasets

a) ML				
Populations	ANA	NKAR	AGA	WAC
ANA	–	0.0037	0.0015	0.0022
NKAR	0.0239	–	0.0123	0.0113
AGA	0.0107	0.0738	–	0.0007
WAC	0.0149	0.0572	0.0062	–
Overall F_{ST}=0.0323, P=0.002				
b) YB				
Populations	ANA	NKAR	AGA	WACOO
ANA	–	0.0537	0.0158	0.0145
NKAR	0.2701	–	0.0405	0.0303
AGA	0.0908	0.1987	–	0.0031
WACOO	0.0851	0.1602	0.0176	–
Overall F_{ST}=0.145, P=0.0001				

Nei's D genetic distance is presented in the upper triangle, and allele-based F_{ST} values in the lower triangle

to any of the candidate populations. Thirty-one individuals in the ML dataset and 40 in the YB dataset were assigned to the populations from which they were sampled. Simulations were performed for the individuals that were not assigned to the populations from which they were sampled (22 and 16 individuals in the ML and YB datasets, respectively). Fifteen out of these 22 ML individuals were assigned with high probability to the populations from which they were sampled, making the number of "correct" assignments in the ML dataset 46 (about 75 %). Similarly, 12 out of the 16 YB individuals were assigned with high probability to the populations from which they were sampled, making the number of "correct" assignments in the YB dataset 52 (about 85 %).

Seven individuals from the ANA population of ML have been allocated to WAC with high P value support from the simulations. In the case of YB, two individuals from ANA have been allocated to AGA, and one to WAC; one individual from NKAR has been allocated to WAC, all with high P value support. Note that all the "misallocations" are among populations close to each other geographically.

Discussion

Operating on the premise that there is a causal relationship between factors that affect dispersal ability, gene flow, and population genetic structure, this study examines whether a difference in wingspan has any effect on population genetics of a species. Using two butterfly species from the Western Ghats of southern India, the AFLP technique was employed to compare patterns in population genetic structure.

Since fragments are amplified in a non-specific manner during the AFLP protocol, it was essential to establish that both datasets can be compared meaningfully. To this end, a comparison of the genetic diversity profiles of both datasets was performed. The overall genetic diversity was very similar in both species. The similarity implies that the differences in population genetic structure between these species are not an artifact of different genetic diversities. The values obtained for genetic diversity in this study (expected heterozygosity, 0.143 in the ML dataset and 0.158 in the YB dataset) are also comparable to values usually obtained globally. When AFLPs are used, H_e values can range from 0.03 in the butterfly *Pieris melete* (Takami et al., 2004), and the moths *Thaumetopoea pityocampa* (Salvato et al. 2002) and *Cydia pomonella* (Thaler et al. 2008), to 0.27 in the moth *Heliothis subflexa* (Groot et al. 2011) and the butterfly *Atrytonopsis* (Leidner and Haddad 2010). This is an important observation, indicating that sampling from a part of the species' range has still captured enough genetic diversity to allow a comparison between the two species. The results of each analysis performed can now be examined in turn (Table 4).

In the spatial autocorrelation analyses, the first distance class showed significant positive spatial autocorrelation, which is expected under a scenario of restricted dispersal. Moving beyond 150 km (the first distance class), there is an interesting shift: significant negative spatial autocorrelation is observed, with a higher r value for ML than YB. This implies that individuals geographically close to each other are more dissimilar than expected at random. Such a sudden shift at around 150 km may be because of the sampling design: this may imply positive correlation values within each sampling group, which become negative when more than one sampling group is considered. The shift is more dramatic in ML: from lower positive r values to higher negative r values. Overall, the higher r values for YB in the first distance class and higher negative r values in ML in the other four distance classes both imply that YB has a more geographically clumped genetic distribution. The result is corroborated by the IBD analysis, where YB has a much higher correlation between genetic and geographic distances than ML.

Even though the sPCA global tests are significant, and indicative of positive spatial autocorrelation, there is no geographic clumping of similar sPCA eigenvalues. This suggests that there is gene flow in both species across the sampling area. While the scores of Gtest cannot be directly compared because they are based on different connection networks, the higher sPCA eigenvalues in YB indicate a higher Moran's I, implying a higher extent of spatial autocorrelation, or more clustering over space, in YB.

Genetic differentiation analyses show that the populations of both species are only weakly differentiated, but YB

Table 4 Comparison of population genetic analyses performed for ML and YB

Analysis	Support for hypothesis
Spatial autocorrelation	r is 2.7 times higher for YB
IBD	Mantel's r 14.3 times greater in YB
sPCA	P value of G test more significant for YB; also, decomposition of eigenvalues had higher Moran's I in YB
Band-based F_{ST}	F_{ST} of YB 2.7 times greater
Allele-based F_{ST}	F_{ST} of YB 4.5 times greater
AMOVA	Variation between populations explained 2.7 times more genetic variation in YB
Assignment tests	1.25 times higher percentage of correct assignment in YB

populations are differentiated to a greater extent than ML populations—the F_{ST} and Nei's D values were higher, and AMOVA results showed that a higher proportion of genetic variation was explained by differences between groups in YB. Differentiation was maximal between populations that are furthest apart (NKAR and AGA) in ML; but, NKAR and ANA are maximally differentiated in YB, with the distance between NKAR and AGA being second highest. Differentiation being lowest between WAC and ANA for both makes intuitive sense, as these are adjacent populations.

The values obtained for population genetic differentiation in the Lepidopteran species, especially butterflies, are generally low (Neve 2009). In a synthesis study on the population genetics of 87 European butterflies, population differentiation values (F_{ST} and its derivatives G_{ST}, or θ) in these studies have a median of 0.053 (0.063 when only Satyrines are considered). Among the Satyrines, the lowest F_{ST} of 0.005 was observed in *Coenonympha pamphilus*, a very common widespread species; the highest F_{ST} (0.291) was observed in *Erebia epiphron*, a montane specialist species. Even when AFLPs are used, F_{ST} values range from 0.006 in *Heliothis virescens* (Groot et al. 2011) to 0.37 in *C. pomonella* (Timm et al. 2006). The values obtained in this study (0.03 for ML and 0.145 for YB) are thus within the range of population differentiation values usually observed in Lepidoptera.

Assignment tests were carried out to trace the source population of each individual. Such tests are considered accurate estimators of dispersal (Berry et al. 2004), because of their potential to trace the source populations of individuals. In this study, there was a higher percentage of correct assignment in YB. There were also fewer allocations to populations other than the one the individual was sampled from, which implies a dispersal event. The low genetic differentiation observed between the ANA and WAC populations of ML is also corroborated in the assignment tests, with seven ML individuals from ANA allocated to WAC with high probability. YB individuals from ANA have been assigned to both AGA and WAC. This implies dispersal between adjacent populations in both species, with more "wrong" assignments in ML, altogether indicating higher dispersal in ML than YB. However, given the moderate population structure exhibited by both species, the assignment test used here might not be a good indicator of migration. This pattern needs to be rigorously tested with larger sample size.

The morphological analysis performed, a comparison of wing aspect ratios, showed that ML, with a lower wing aspect ratio, can be expected to be a better, faster flier. This adds support to the hypothesis that ML is a better disperser than YB. Thus, the results from all the analyses clearly show that YB exhibits signatures of restricted dispersal, while ML has a comparatively homogenous genetic structure.

However, unless capture mark recapture studies are performed, it is difficult to analyze whether the geographic scale at which the study was carried out was large enough to encompass their dispersal distances. Both species are habitat generalists, and were found at relatively high densities in almost all the sites that were visited. YB abounds in grassy patches in forest clearings, while ML is found in shaded patches of forest. ML is cryptic and difficult to spot, but individuals were recorded at high densities by using traps.

Such field-based studies have not been done in India, but we can look at the dispersal distances of species from the same subfamily (Satyrinae) from other countries, where this information is available (Appendix 1 of the paper, Sekar 2012). For instance, the highest mean distance dispersed (males) is 600 m in *Erebia epipsodea* (Brussard 1970), and the mean distance dispersed (females) is 251 m in *Aphantopus hyperantus* (Billeter et al. 2003). The longest observed dispersal event recorded in field studies is 2100 m in *Maniola jurtina* among males (Schneider et al. 2003) and 970 m in *A. hyperantus* among females (Billeter et al. 2003). The distances that have been considered in the current study are many times the maximal distance from this compilation.

In many studies, butterfly mobility has been established as a reliable predictor of species response to habitat fragmentation and climate change. For example, life history traits, especially wingspan, have been shown to predict species response to habitat isolation (Barbaro and van Halder 2009; Ockinger et al. 2010). Species with low mobility, a narrow feeding niche, and low reproduction were most strongly affected by habitat loss. The migration and colonization of new habitats is more likely in species with a higher wingspan and hence

higher dispersal ability (Dennis et al. 2000). In the fragmented British landscape and in continental Europe, butterflies with higher mobility are doing better than those with low and intermediate mobility (Thomas 2000). In such a context, understanding the drivers of butterfly mobility and how they affect genetic structure in butterflies is important.

This study adds to the very few studies that try to establish a relationship between wingspan and dispersal ability, and hence genetic structure. The strength of the study lies in the comparative approach, especially due to the species used: closely related sympatric species, with similar ecologies and dispersal traits, that differ only with respect to their wingspan. It also confirms the utility of AFLPs in population genetic studies: the genetic diversity and differentiation indices of both study species are comparable to other studies, in spite of the study being restricted to a small geographic area. The population genetic patterns of the two species have been compared using six different analyses methods, generating concordant results. The conclusion of the study supports the hypothesis of wingspan affecting dispersal ability in butterflies; but caution must be exercised while extrapolating the result to other species pairs, or as a universal truth. More studies need to be carried out to explicitly test the relationship between wingspan, dispersal ability, and gene flow, and robust comparisons are the way forward in testing this interesting hypothesis.

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