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RESEARCH

Morphological Outcomes of Gynandromorphism in *Lycaeides* Butterflies (Lepidoptera: Lycaenidae)

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ABSTRACT. The genitalia of male insects have been widely used in taxonomic identification and systematics and are potentially involved in maintaining reproductive isolation between species. Although sexual selection has been invoked to explain patterns of morphological variation in genitalia among populations and species, developmental plasticity in genitalia likely contributes to observed variation but has been rarely examined, particularly in wild populations. Bilateral gynandromorphs are individuals that are genetically male on one side of the midline and genetically female on the other, while mosaic gynandromorphs have only a portion of their body developing as the opposite sex. Gynandromorphs might offer unique insights into developmental plasticity because individuals experience abnormal cellular interactions at the genitalic midline. In this study, we compare the genitalia and wing patterns of gynandromorphic Anna and Melissa blue butterflies, *Lycaeides anna* (Edwards) (formerly *L. idas anna*) and *L. melissa* (Edwards) (Lepidoptera: Lycaenidae), to the morphology of normal individuals from the same populations. Gynandromorph wing markings all fell within the range of variation of normal butterflies; however, a number of genitalic measurements were outliers when compared with normal individuals. From these results, we conclude that the gynandromorphs' genitalia, but not wing patterns, can be abnormal when compared with normal individuals and that the gynandromorphic genitalia do not deviate developmentally in a consistent pattern across individuals. Finally, genetic mechanisms are considered for the development of gynandromorphism in *Lycaeides* butterflies.

Key Words: developmental plasticity; genitalia; gynandromorph, local outlier factor; *Lycaeides anna*; *Lycaeides melissa*

Male insect genitalia have been extensively studied due to the role they potentially play in reproductive isolation between species and for their utility in taxonomy and morphological systematics (Scudder 1971; Shapiro and Porter 1989; Hosken and Stockley 2004; Eberhard 1985, 2010, 2011). Many hypotheses have been put forth to explain the evolution of genitalic morphology, with sexual selection thought to play a key role (Hosken and Stockley 2004). An alternative and also complementary context for investigating the evolution of phenotypic variation in sexual traits is provided by evolutionary developmental biology (Brakefield et al. 2003, West-Eberhard 2003). Developmental plasticity can create dramatic phenotypic variation that is subject to subsequent selective pressures. For example, variation in eyespot wing patterns in *Bicyclus anynana* (Butler) butterflies (Nymphalidae) arises through developmental plasticity in the expression of the Distal-less (Dll) regulatory gene and can subsequently be fixed through selection (Brakefield et al. 1996, Beldade and Brakefield 2002). A similar developmental perspective has not been widely applied to genitalic morphology outside of model organisms.

Bilateral gynandromorphs are developmental aberrations in which an individual's body is half male and half female, with cells on either side of the bilateral split containing alternate sex chromosome combinations (Narita et al. 2010). For example, in most bilateral gynandromorph butterflies, one half of the body contains ZZ sex chromosomes (male) while the other half possesses ZW sex chromosomes (female). In addition, many individuals can develop as mosaic gynandromorphs, with only some portions of their bodies developing as the opposite sex. The study of both bilateral and mosaic individuals offers potential insights into the range of phenotypes that may be produced by developmental plasticity in genitalic development because individuals experience unusual genetic and hormonal environments (both male and female) at the genitalic midline compared with normal individuals. It is worth noting that hormones have only recently been thought to be important in insect sex-determination; however, gynanrdromorphs are considered some of the best evidence for cell-autonomous (i.e., genetically predetermined) sex determination (Bear and Monteiro 2013).

Gynandromorphism is a rare phenomenon in both natural and laboratory environments (Scriber and Evans 1988); however, many gynandromorphs have been described, especially among the arthropods (reviewed by Narita et al. 2010). In addition to arthropods, gynandromorphs have been documented in birds (Agate et al. 2003, Zhao et al. 2010), reptiles (Mitchell and Fouquette 1978, Krohmer 1989), amphibians (Zug 1987), fish (O'Farrell and Peirce 1989), and mammals (Hollander et al. 1956, Renfree et al. 1987). In this study, we compare the genitalia and wing patterns of five gynandromorphic Melissa blue butterflies, *Lycaeides melissa* (Edwards) (Lepidoptera: Lycaenidae), and one gynandromorphic Anna blue butterfly, *L. anna* (Edwards) (formerly *L. idas anna*), to the morphology of normal individuals both wild-caught and reared from wild populations. As is typically the case in genitalic studies of insects, we focus on male morphology due to the ease of measuring sclerotized features. In particular, we asked if morphological features relatively near and far from the midline (i.e., genitalia and wing patterns) were divergent from normal individuals. In addition, we asked if midline features in gynandromorphic individuals would be stochastically or consistently aberrant. If all gynandromorphs differ from normal individuals in the same manner, this could suggest little room for developmental plasticity in genitalia. In contrast, if all gynandromorphic genitalia vary in different ways, this could be consistent with greater room for plasticity or stochasticity in development, and more complex developmental architecture.

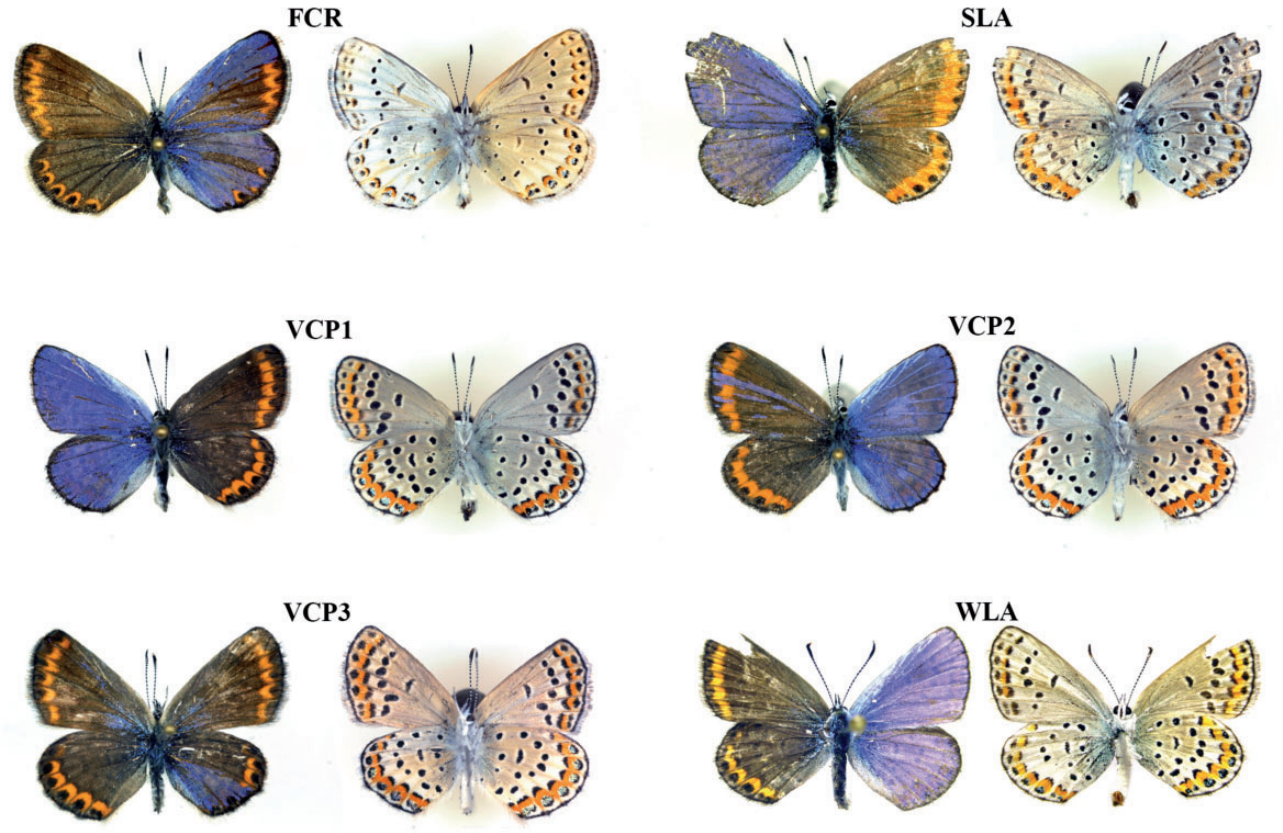

Fig. 1. Dorsal and ventral wing patterns of six gynandromorphic *Lycaeides* butterflies from four populations [Fall Creek (FCR), Silver Lake (SLA), Verdi (VCP), and Washoe Lake (WLA)]. On the dorsal surface, males are blue, while females are brown and orange.

Materials and Methods

Several distinct *Lycaeides* lineages are found throughout North America, including *L. anna* and *L. melissa* (Nice et al. 2005, Gompert et al. 2008), with most taxa having an extensive history of hybridization and genetic admixture (Gompert et al. 2014). The history of hybridization between *L. anna* and *L. melissa* is particularly noteworthy, as gene flow between these lineages led to the formation of multiple hybrid species (Gompert et al. 2006, Nice et al. 2013). Ecological restrictions on mating between *L. melissa* and *L. anna* have been hypothesized (Scholl et al. 2012), and current gene flow is likely low or absent. In the summers of 2011 and 2012, six gynandromorphic *Lycaeides* butterflies were either captured from wild populations or reared in a laboratory at the University of Nevada, Reno, from eggs obtained from wild-caught females (Fig. 1). See Table 1 for collection and locality information, and note that we refer to the three gynandromorphs from Verdi (VCP) in chronological order (VCP1 = individual collected on July 7, 2012; VCP2 = July 11, 2012; VCP3 = July 28, 2012). Three of the individuals were bilateral gynandromorphs based on wing coloration [Silver Lake (SLA), Washoe Lake (WLA), and VCP1], while the other three individuals were mosaic gynandromorphs [Fall Creek (FCR), VCP2, and VCP3; Fig. 1]. All specimens were pinned before genitalia were dissected and photographed.

We compared male genitalic morphology of each gynandromorph to the genitalia of normal males from each gynandromorph's natal population. We measured wild-caught and laboratory-reared normal males to characterize the range of natural variation in male morphology in each population, including variation due to phenotypic plasticity (in laboratory vs. wild comparisons). Specifically, we measured 15 wild-caught and 12 laboratory-reared males from WLA, 15 wild-caught and 13 laboratory-reared males from FCR, 15 wild-caught and 15 laboratory-reared males from VCP, and 19 wild-caught and 15 laboratory-reared males from SLA. We removed the posterior-most abdominal segments of each individual and submerged them in hot (~95°C), 5M potassium hydroxide for 15 min. The soft tissues dissolve during this process but sclerotized structures become easy to dissect. Each genitalia was positioned under a coverslip such that the valvae were pushed to the side and the falx and aedeagus could be viewed clearly. We dissected and imaged each male genitalia using a Leica stereo-microscope and camera. We used a stage micrometer to standardize each measurement. Genitalia are stored in labeled micro vials in glycerin and housed at Utah State University.

We used ImageJ (http://rsbweb.nih.gov/ij/) to take five measurements of the right uncus: forearm length (F), the width of the forearm midpoint (FM), elbow width (E), humerulus length (H), and uncus length (U) (Fig. Supp S1). If the right uncus was damaged or obscured, we measured the left side (there is no fluctuating asymmetry in *Lycaeides* male genitalia; L.K.L., unpublished data). We chose to measure these five aspects of the uncus because they have been used to differentiate North American *Lycaeides* species (Nabokov 1943, 1944, 1949; Lucas et al. 2008). Furthermore, male butterflies use the falx to internally clasp onto the female during mating, and Gompert et al. (2012) demonstrated they are associated with reproductive isolation. We also measured the length and width of the aedeagus [aedeagus length (AL) and aedeagus width (AW), respectively] for those populations whose gynandromorph had an aedeagus (WLA and VCP; Fig. 2).

We also compared wing pattern morphology of each gynandromorph to wing patterns of normal males and females from each gynandromorph's respective population. Specifically, we measured 13 wild-caught and 8 laboratory-reared males and 22 wild-caught females from WLA, 10 wild-caught and 11 laboratory-reared males and 24 wild-caught females from FCR, 15 wild-caught and 14 laboratory-reared males and 29 wild-caught females from VCP, and 15 wild-

Table 1. Specimen information for the six gynandromorphic *Lycaeides* butterflies

Date	Population	Latitude	Longitude	Species	Host plant	Laboratory/wild	Morphology
July 19 2011	Washoe lake (WLA)	38.65	118.82	*Lycaeides melissa*	*Astragalus canadensis*	Captured	Bilateral
June 14 2012	Silver lake (SLA)	39.65	119.93	*L. melissa*	*A. canadensis*	Captured	Bilateral
July 7 2012	Verdi (VCP1)	39.51	120.00	*L. melissa*	*Medicago sativa*	Reared	Bilateral
July 8 2012	Fall creek (FCR)	39.38	120.67	*Lycaeides anna*	*Lotus nevadensis*	Reared	Mosaic
July 11 2012	Verdi (VCP2)	39.51	120.00	*L. melissa*	*M. sativa*	Reared	Mosaic
July 28 2012	Verdi (VCP3)	39.51	120.00	*L. melissa*	*M. sativa*	Reared	Mosaic

Laboratory/wild refers to whether the butterflies were captured from wild populations or reared in the laboratory (from eggs laid by wild-caught females), and morphology describes whether individuals are bilateral or mosaic gynandromorphs.

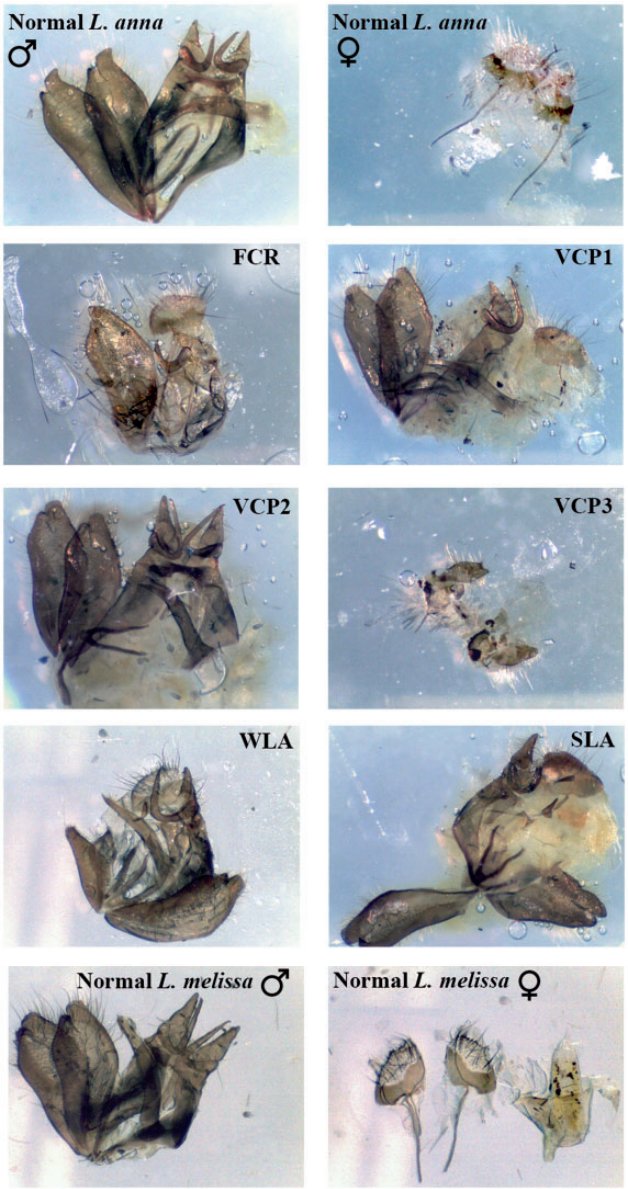

Fig. 2. The genitalia of six gynandromorphic butterflies are compared with the genitalia of normal male and female *Lycaeides anna* and *Lycaeides melissa* butterflies from four populations (FCR, SLA, VCP, and WLA).

caught and 13 laboratory-reared males and 28 wild-caught females from SLA. We removed both hind wings from each gynandromorph and one hind wing from each normal individual and photographed each under a coverslip using a Leica stereo-microscope and camera. We used a stage micrometer to standardize each measurement. Wings are stored in glassine envelopes and housed at Utah State University. We used ImageJ to measure the area (in mm) of each wing and 17 ventral wing elements, five aurorae (a2, a3, a4, a5, a6) and 12 black spots: Cu_2(3), M, Sc(3), Sc, Rs, M_1, M_2, M_3, Cu_1, Cu_2+1_A, 2A and c (Fig. Supp S2). We chose to measure the size of these 17 elements because Fordyce et al. (2002) demonstrated that they act as effective mate recognition cues in *Lycaeides* butterflies and can also be used to discriminate among populations and species. We divided each wing pattern element area by its respective total wing area to standardize element size relative to the size of the wing.

To determine if each gynandromorph's male genitalia and wing markings fell within the normal range of variation in their natal population, we first examined each gynandromorph in relation to the normal males from their population in multivariate space. We performed principal components analysis (PCA) with a correlation matrix for each population using the *princomp* function in R (R Development Core Team, 2013). For genitalic PCAs, the seven genitalic measurements described earlier (F, U, H, E, FM, AL, and AM) were used as input data; however, AL and AM were excluded from populations whose gynandromorph did not have an aedeagus. The 17 standardized wing element measurements described earlier were used as input data for all wing marking PCAs.

In order to examine each genitalic measurement of the gynandromorphs relative to normal individuals from their natal populations, we conducted outlier analyses. In this study, we follow Hawkins (1980) in defining an outlier as "an observation which deviates so much from the other observations as to arouse suspicions that it was generated by a different mechanism." Outlier analyses work under Hawkins's assumption that outliers are created under a different mechanism relative to the other data and thus look for data that are in areas of low density relative to other data in the same proposed distribution (Aggarwal 2013). In contrast, extreme value statistics (e.g., Grubbs outlier tests; Grubbs 1950) only identify points that are extreme relative to a measure of central tendency and do not specifically follow Hawkins's assumption (Aggarwal 2013). Because our goal in this study was to determine if the genitalia of a few individuals were different from the majority of the entire population due to a differing developmental mechanism (i.e., gynandromorphism), we chose to utilize outlier analysis instead of extreme value analysis. For each genitalic measurement, we performed local outlier factor (LOF) identification (Breunig et al. 2000), which calculates an outlierness score for every point in the data based on the distance of each point to its closest neighboring points (k). Most points have LOF scores that cluster around one, while outliers have LOF scores greater than one because they reside in low-density areas in the distribution. LOF identification was performed with the DMwR package in R (Torgo 2010) with $k = 5$. Kernal density estimates of LOF scores were plotted using the *density* function in R. Because LOF scores do not indicate the directionality of an outlier (i.e., whether the individual is abnormally larger or smaller than other individuals), histograms were also plotted using R.

Table 2. Summary statistics for the genitalic principal component analyses (PCAs), including the proportion of variation explained for each principal component (PC), the cumulative variation explained, and the loadings of each genitalic measurement on each PC

Population	PC	Proportion variation	Cumulative variation	F	H	U	E	FM	AL	AW
FCR	1	0.419	0.419	−0.54	−0.56	−0.52	−0.32	−0.15	NA	NA
	2	0.253	0.672	0.15	0.21	0.20	−0.60	−0.73	NA	NA
	3	0.173	0.846	0.31	0.00	−0.58	0.54	−0.52	NA	NA
SLA	1	0.345	0.345	−0.66	−0.59	−0.41	−0.16	0.12	NA	NA
	2	0.267	0.612	0.28	−0.14	−0.24	−0.56	−0.73	NA	NA
	3	0.187	0.798	0.00	−0.33	0.75	−0.53	0.23	NA	NA
VCP	1	0.383	0.383	−0.52	−0.49	−0.34	0.00	−0.21	−0.53	−0.22
	2	0.236	0.619	−0.14	0.19	−0.36	0.59	0.47	−0.21	0.45
	3	0.140	0.759	0.00	0.24	−0.56	−0.40	0.47	0.19	−0.45
WLA	1	0.370	0.370	−0.52	0.00	−0.48	0.33	0.31	−0.51	−0.18
	2	0.228	0.599	−0.21	−0.63	−0.13	−0.32	−0.58	−0.24	−0.21
	3	0.152	0.751	−0.16	−0.38	0.00	0.35	0.00	0.00	0.83

See text for description of genitalic measurements.

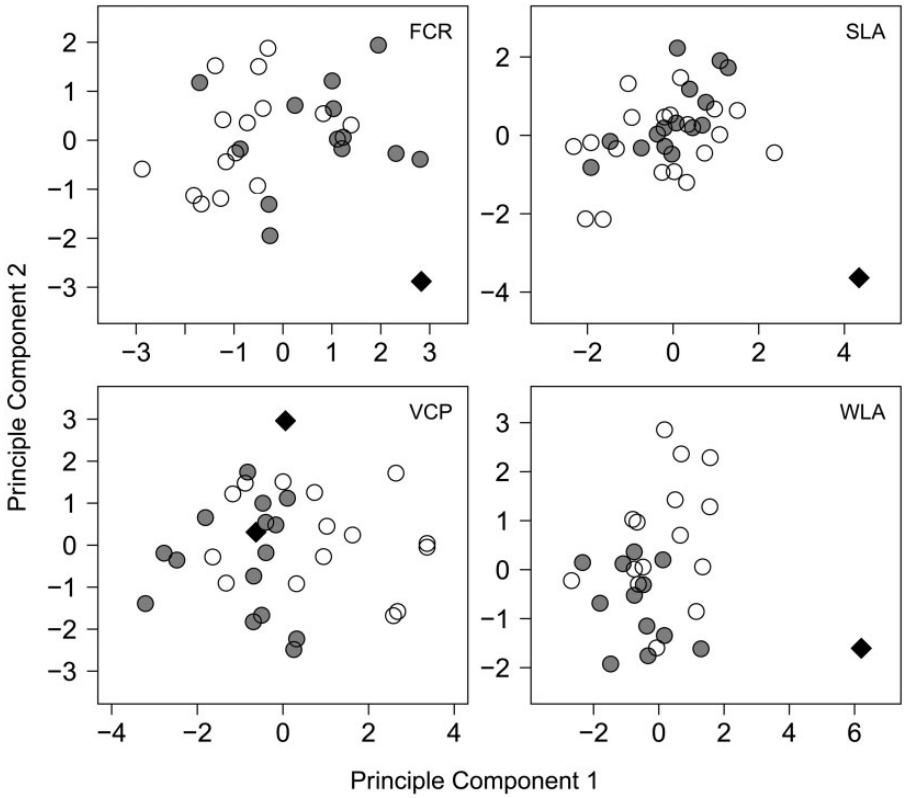

Fig. 3. The first two principal component (PCs) from the genitalic PCA are plotted in order to compare the genitalia of the gynandromorphs (black diamonds) to normal male genitalia from wild-caught (open circles) and laboratory-reared (grey circles) individuals from four populations (FCR, SLA, VCP, and WLA).

Results

Of the six gynandromorphs examined in this study, one individual had genitalia similar to normal males (VCP2), and one individual had genitalia similar to normal females (VCP3; Fig. 2). The other four individuals had genitalia that were half male and half female, with two containing an aedeagus (VCP1 and WLA), and two missing an aedeagus (FCR and SLA). The first two genitalic principal components (PC) explained at least 59.9% of the morphological variation for all sites (Table 2). Four gynandromorphs, FCR, SLA, VCP1, and WLA, fell outside the cluster of normal males when comparing the first two PCs (Fig. 3). In contrast, VCP2 was not qualitatively distinct from normal males when plotted in PC space (Fig. 3). Interestingly, the genitalia of VCP3 were phenotypically female, so this individual was excluded from all genitalic analyses. Although the first two PCs in the analysis of ventral wing markings also explained a high proportion of morphological variation in all sites (at least 61.2%; Table 3), none of the gynandromorphs were distinguishable from normal males or females in PC space (Fig. 4).

For the four gynandromorphs that were distinguishable from normal males in the genitalic PCA (Fig. 3), a number of genitalic measurements had high LOF scores (Fig. 5). The FCR gynandromorph's forearm length, humerulus, and uncus measurements were outliers relative to normal males, while the forearm length and midpoint measurements were outliers for the SLA gynandromorph. The humerulus and uncus measurements were also outliers for VCP1, but all of the measurements for the VCP2 gynandromorph were not outliers (this individual was not distinguishable from normal males in the genitalic PCA). The WLA individual was an outlier for the forearm length and midpoint, humerulus,

Table 3. Summary statistics for the wing marking PCAs, including the proportion of variation explained for each PC, the cumulative variation explained, and the loadings of each wing measurement on each PC

Population	PC	Proportion variation	Cumulative variation	a2	a3	a4	a5	a6	$Cu_2(3)$	M	Sc(3)	Sc	Rs	M_1	M_2	M_3	Cu_1	Cu_2+1_A	2A	c
FCR	1	0.381	0.381	−0.22	−0.20	−0.19	−0.21	−0.22	−0.25	−0.26	−0.29	−0.26	−0.20	−0.22	−0.30	−0.23	−0.30	−0.30	−0.27	−0.15
	2	0.231	0.612	−0.33	−0.40	−0.40	−0.39	−0.37	0.14	0.18	0.12	0.29	0.20	0.19	0.13	−0.74	0.14	0.16	0.10	0.57
	3	0.091	0.703	0.21	0.16	0.11	0.17	−0.18	−0.38	−0.40	−0.15	0.11	0.40	0.65	0.30	0.31	−0.33	−0.19	−0.47	0.32
SLA	1	0.522	0.522	−0.17	−0.15	−0.18	−0.18	−0.16	−0.24	−0.29	−0.28	−0.28	−0.28	−0.25	−0.26	−0.26	−0.31	−0.30	−0.25	−0.20
	2	0.197	0.720	−0.36	−0.44	−0.39	−0.41	−0.40	0.20	−0.26	0.10	0.29	0.13	0.22	0.19	0.19	−0.16	0.16	−0.11	−0.61
	3	0.057	0.777	−0.14	0.24	0.23	0.23	−0.34	−0.33	−0.37	0.18	0.15	0.26	0.35	−0.11	−0.53	−0.23	−0.21	0.54	−0.16
VCP	1	0.463	0.463	−0.22	−0.23	−0.22	−0.22	−0.18	−0.24	−0.26	−0.23	−0.21	−0.25	−0.24	−0.30	−0.27	−0.29	−0.27	−0.24	−0.21
	2	0.171	0.634	−0.32	−0.34	−0.40	−0.43	−0.40	−0.29	−0.26	−0.47	0.25	0.19	0.25	0.31	0.19	0.13	0.16	−0.21	0.14
	3	0.069	0.703	−0.14	−0.32	−0.23	−0.11	0.32	0.47	0.23	−0.14	−0.27	0.18	0.33	0.15	0.37	0.22	−0.28	0.27	−0.28
WLA	1	0.448	0.448	−0.20	−0.17	−0.22	−0.22	−0.19	−0.27	−0.27	−0.21	−0.27	−0.22	−0.24	−0.31	−0.28	−0.32	−0.27	−0.21	−0.19
	2	0.195	0.643	−0.31	−0.39	−0.40	−0.39	−0.37	−0.32	0.29	0.54	0.28	0.24	0.22	0.14	0.13	0.15	0.19	0.34	−0.21
	3	0.067	0.711	−0.19	−0.30	0.14	0.25	0.17	−0.50	−0.21	−0.37	0.18	0.15	−0.48	−0.11	0.30	0.20	−0.25	0.12	−0.68

See text for description of wing measurements.

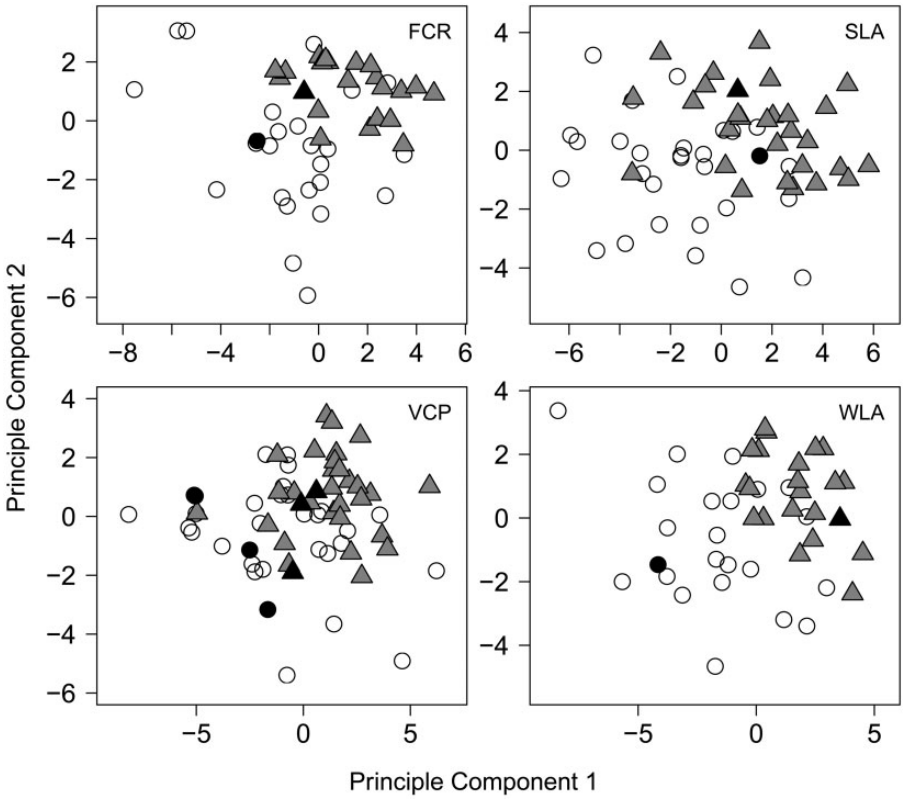

Fig. 4. The first two PCs from the wing pattern PCA are plotted in order to compare the wing patterns of gynandromorphic female wings (black circles) to normal female wings (white circles) and gynandromorphic male wings (black triangles) to normal male wings (grey triangles) from four populations (FCR, SLA, VCP, and WLA).

uncus, and elbow measurements. All gynandromorphs from populations with an aedeagus (VCP and WLA) were not outliers for the AL and AW measurements. Despite the morphological variation among gynandromorphs, the directionality of outliers was fairly consistent across individual measurements (e.g., all gynandromorphs that were outliers for the forearm length measurement had smaller forearm lengths than normal males; Fig. 5; Fig. Supp S3). The only variation in directionality was found in humerulus outliers, with the FCR humerulus smaller than normal males and the VCP1 and WLA humeruluses larger than normal males.

Discussion

Although early studies of gynandromorphs were initially focused on understanding genetic pathways in *Drosophila* Fallén (e.g., Morgan 1914, Morgan and Bridges 1919, Dobzhansky 1931, Garcia-Bellido and Merriam 1969), researchers have recently suggested that gynandromorphs may be useful models for the study of sex determination (Agate et al. 2003, Narita et al. 2010, Zhao et al. 2010, Clinton et al. 2012), modularity, and evolvability (Yang and Abouheif 2011). Previous studies describing gynandromorphs have frequently relied on visual descriptions of individuals and rarely provided statistical analyses to compare gynandromorphs to normal individuals from the same population (e.g., Kusnezov 1926, Dobzhansky 1931). In this study, we statistically compared the genitalia and wing patterns of *Lycaeides* gynandromorphs to the morphology of normal males from the same populations.

Although none of the gynandromorphs' wing patterns fell outside the range of variation found in normal males (Fig. 4), four of the six

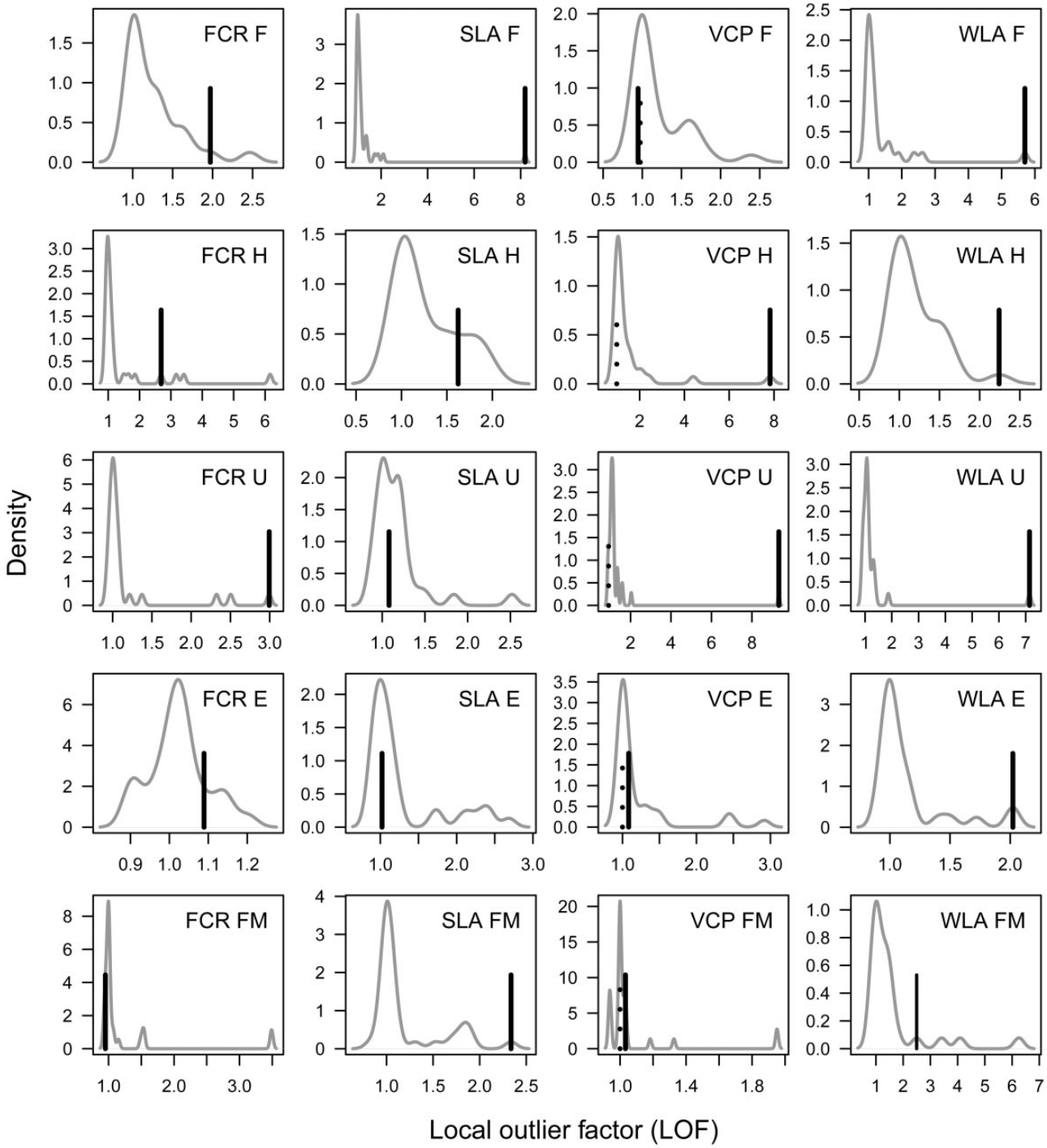

Fig. 5. Kernal density estimates of local outlier factors (LOF) are displayed for five genitalic measurements [forearm length (F), humerulus (H), uncus (U), elbow (E), and width of the forearm midpoint (FM)] for butterflies from four populations (FCR, SLA, VCP, and WLA). The vertical bars in each figure denote the LOF score for gynandromorph genitalia. For the VCP panels, the solid vertical bars correspond to the genitalia of VCP1 and the dotted vertical bars correspond to the genitalia of VCP2.

gynandromorphs had at least two genitalic measurements that were outliers (Fig. 5). Interestingly, these gynandromorphs were all outliers for different morphological features. For example, the SLA gynandromorph was an outlier for the forearm length and midpoint measurements, while the VCP1 gynandromorph was an outlier for the humerulus and uncus measurements. The genitalic outliers that were larger than normal males (VCP1 and WLA humerulus, WLA elbow width, and SLA and WLA forearm midpoint (FM)) are particularly interesting because one might expect that all of the male genitalic structures measured in the gynandromorphs would undergo a proportional size reduction (as in forearm length and uncus outliers) due to the presence of excess female cells and hormones at the midline. This result suggests that some reproductive structures may have dissimilar plastic developmental responses in different individual gynandromorphs.

A remaining question from this study is whether or not the gynandromorphs' genitalia were functional. We attempted to address this by

placing the wild-caught SLA gynandromorph in a standard oviposition arena that has been used in previous studies examining host use in *Lycaeides* butterflies (Forister et al. 2009, 2013). The SLA gynandromorph made repeated oviposition attempts on its native host (*Astragalus canadensis* L.; Table 1), but was ultimately unsuccessful in laying eggs (J.P.J., pers. obs.). These behaviors suggest that at least the SLA gynandromorph had non-functional genitalia or oviduct, or most likely both, because even *Lycaeides* females that have not been mated will readily lay unfertilized eggs (M.L.F., pers. obs.).

Many possible genetic mechanisms of gynandromorphism have been proposed, including loss of a sex chromosome during mitosis, genetic modification by endosymbionts, and double fertilization of binucleate eggs (Narita et al. 2010). For Lepidoptera, and presumably for the *Lycaeides* bilateral gynandromorphs in this study (SLA, VCP1, WLA; Fig. 1), double fertilization of a binucleate egg is expected to be the most common mechanism of gynandromorphism (Cockayne 1935, Blanchard and Descimon 1988). However, mosaicism can also sometimes occur in Lepidoptera via loss of the W chromosome during a nondisjunction event in later stages of development. W chromosomes typically carry little genetic information and sex determination is thought to be mostly based on the number of Z chromosomes in a cell, with males having two copies and females having one (Scott 1986). The nondisjunction mechanism may be relevant for the mosaic butterflies examined in this study (FCR, VCP2, VCP3).

It has been suggested that most cases of gynandromorphism in butterflies of the genus *Papilio* L. are caused by hybridization between species or subspecies (Scriber and Evans 1988, Scriber et al. 2009). However, this appears to be an unlikely mechanism for gynandromorphs in our system because *L. melissa* and *L. anna* (formerly known as *L. idas anna*) are not sympatrically distributed in the region of our focal populations. Therefore, interspecific matings between *L. melissa* and *L. anna* resulting in gynandromorphs would not be expected. *L. melissa* does form a hybrid zone with the closely related species *Lycaeides idas* (L.) in the Rocky Mountains (Gompert et al. 2010, 2012), so the potential does exist for an interspecific gynandromorph between those two species in other regions.

Finally, increased occurrence of gynandromorphism in butterflies has been linked with exposure to low-dose radiation (Dantchenko et al. 1995). For example, a small number of gynandromorphic pale grass blue butterflies (*Zizeeria maha* (Kollar)) have been found in areas within the Fukushima nuclear accident fallout area in Japan (Hiyama et al. 2013). Over 7,000 *Lycaeides* butterflies have been captured or reared from a number of sites across North America between 2003 and 2014; however, all six of the gynandromorphs in this study were captured or reared in the 16 mo following the Fukushima nuclear accident and none have been captured since (M.L.F., pers. obs.). Although we have no reason to link low-dose radiation exposure with the spatial and temporal concentration of gynandromorphs described in this study, it is intriguing to note that radiation from Fukushima reached the western United States 4 mo prior to the first gynandromorph capture (Thakur et al. 2012).

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