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TAXONOMIC UTILITY OF EGG MICROSCULPTURE: MATERNAL EFFECTS AND VARIATION IN EGGS OF *PARNASSIUS SMINTHEUS* DOUBLEDAY (PAPILIONIDAE)

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ABSTRACT. Egg microsculpture has been used at various phylogenetic levels as an informative morphological character set; in particular, the structure of the micropylar rosette has been used to differentiate between closely related taxa at the species-level, but intraspecific variation in this character set remains largely undocumented. Here we show that the number and shape of elements in the micropylar rosette can vary substantially within a population of a single species and even within individual females. A significant proportion (52%) of this variation is attributable to the source female (but uncorrelated with female size), suggesting that some unknown maternal effect influences micropyle structure. Due to this large intrapopulation variation, the utility of this character set and the taxonomic rank to which it is applied should be evaluated carefully.

Additional key words: micropyle, micropylar rosette, taxonomy, morphology, *Parnassius phoebus*, *Parnassius behrii*

Morphological characters are routinely used to make taxonomic and phylogenetic assignments. When using these traits, the premise is that variation among taxa is sufficiently greater than variation within each taxon, thus allowing distinction. For many characters, it is relatively easy to identify sources of variation that need to be accounted for before making comparisons. For instance sexually dimorphic characters would only be compared within sex. However for other morphological characters, such as egg microsculpture, sources of variability may be more inconspicuous.

Although Lepidopteran egg microsculpture has long been documented and described (e.g. Edwards 1872; Peyron 1909; Döring 1955), the use of this morphological character set in Lepidoptera taxonomy has been relatively limited. For example, Häuser et al. (1993) documented egg microsculpture in Parnassiinae, noting that chorion morphology was informative at the genus and sometimes the species-group level. Building on the efforts of Seamans (1933), Salkeld (1975, 1976) used chorionic microsculpture to differentiate between species-groups of the taxonomically difficult cutworm genus *Euxoa*, but noted that species-level differentiation was generally not possible. More recently, variation in micropyle structure has been applied to differentiate among species of the *Papilio machaon* L., 1758 group (Eitschberger 1993) and the *Parnassius phoebus* (Fabricius, 1793) group (Shepard & Manley 1998). Harbich (1996, 1997) and Danner et al. (1998) also used egg microsculpture to elevate several *Hyles* (Sphingidae) taxa to species level, although these taxa were subsequently re-synonymized (Kitching & Cadiou 2000).

Despite the purported utility of egg microsculpture in sibling species taxonomy, intraspecific variation of micropyle structure remains poorly documented. The purpose of this paper is to examine the variation in structure of the micropylar rosette in a population of *Parnassius smintheus* Doubleday, 1847 (Papilionidae). Of the potential variation in overall egg microsculpture, we focused specifically on the micropyle since this structure was the most obviously variable and it has previously most often been used to make taxonomic inferences in Papilionidae.

MATERIALS AND METHODS

We collected 11 female butterflies from two large contiguous populations (Meadows P & Q, see Matter et al. 2004) along Jumpingpound Ridge, Kananaskis, Alberta, Canada (51°57'N, 114°54'W, ~2100 m). Kananaskis Country butterflies were removed on four dates between 8 and 23 Aug 2002. Upon capture, individual females were placed in glassine envelopes and housed at The University of Calgary's Biogeosciences Institute (~1400 m) under ambient conditions. Under these conditions females will continue to lay eggs (Matter et al. 2006).

We examined the morphology of eggs produced by individual females. Between 11 and 20 eggs were examined for each of the 11 females. For each egg we counted the number of elements around the micropyle (Fig. 1). Additionally we measured the forewing length

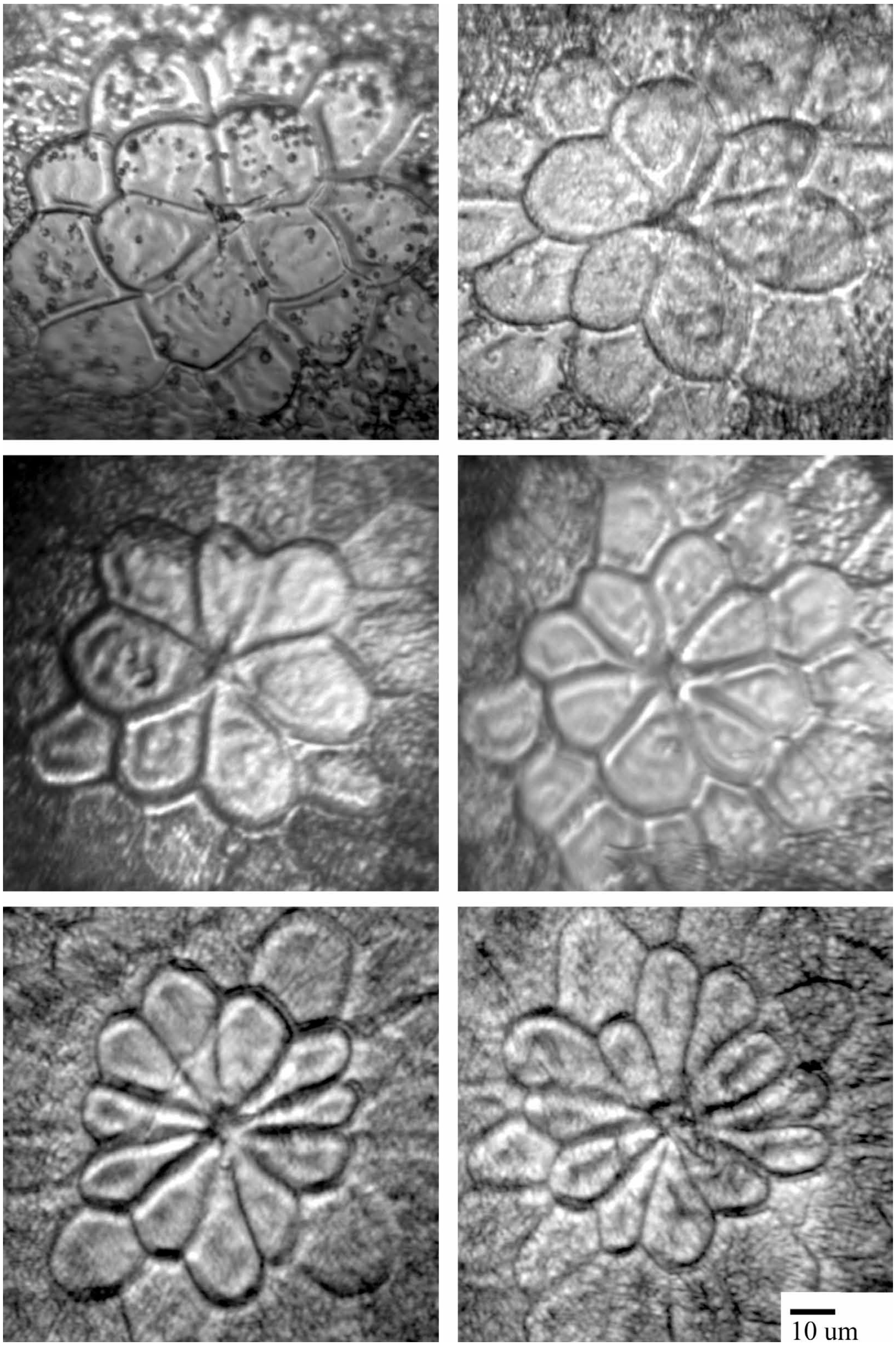

FIG. 1. Variation in micropyle rosette structure of *P. smintheus* eggs, produced by two females. Eggs in the top and middle rows were produced by one female, those in the bottom row by another. The images are centered on the micropyle, with polygon- to pie-shaped elements forming the surrounding rosette. Eggs were photographed at 240 × magnification using a Lumenera Infinity 1 digital microscope camera, mounted on a Leica M165C dissecting microscope.

(base to apex) of each female to determine if the number of elements was related to female size. The number of elements around the micropyle was analyzed using a generalized linear model assuming the number of elements had a Poisson distribution. Mean number of elements per egg was regressed against the forewing length of each female to determine if the number of elements was related to female size. To determine if the number of elements around the micropyle varies with egg size, we compared the diameter of eggs to the number of elements around the micropyle. Because the number of elements consists of count data, a Poisson regression was used. Ten additional eggs from six females were used for this analysis. Diameter of the egg was measured at its widest point using the program ImageJ (Abramoff et al. 2004). Digital images for measurements were captured at 60 × magnification using a Leica EC3 camera mounted on a Leica M80 dissecting microscope.

RESULTS

Females accounted for a significant amount of variation in the number of elements surrounding the micropyle of *P. smintheus* eggs (χ^2 = 18.5, df = 10, P = 0.04; Fig. 2). The number of micropylar elements ranged from 5 to 12, (mean 7.6 ± 1.6 (SD)). Element shape varied according to the number of elements, with five- and six-element rosettes exhibiting more polygonal rather than wedge-shaped elements (Fig. 1). Over 52% of the deviance (variation) in the number of elements around the micropyle was explained by the mother. We found no significant relationship between female forewing length and number of elements around the micropyle ($F_{1,8}$ = 0.14, MSE= 0.31, P = 0.72). Similarly, there was no relationship between egg diameter and the number of micropylar elements (χ^2 = 0.04, df = 1, P = 0.84).

DISCUSSION

The egg surface of *Parnassius* species is highly sculptured compared to other Papilionidae, likely a result of the thick chorion evolved to protect the overwintering egg or pharate larva from predators, parasitoids and adverse environmental conditions (Häuser et al. 1993). The number of elements surrounding the micropyle is due in part to maternal effects; individual females produce eggs with a characteristic mean number, but also show egg to egg variability. We suspect that other characters related to egg morphology show similar trends. Before using such characters in a taxonomic or phylogenetic analysis, variation due to maternal effects should be accounted for. In lieu of this, eggs should be collected from many different individuals to overcome this source of variability. Furthermore, infraspecific geographic variation remains undocumented.

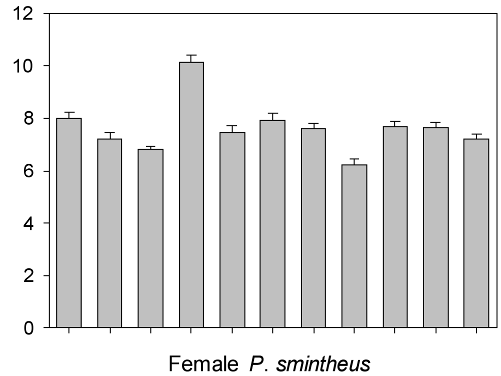

FIG. 2. Variation among female *P. smintheus* in the mean number of micropyle rosette elements. Means are shown ± SE.

The most recent taxonomic revision of the North American *Parnassius phoebus* complex (Shepard & Manley 1998) relied heavily on the morphology of the micropylar rosette as a diagnostic character. Although we are not attempting to discredit the taxonomic assignments of Shepard & Manley (1998), additional work clearly is needed to better understand the taxonomy and biogeography of the North American *P. phoebus* group, since the intrapopulation variation in egg microsculpture of *P. smintheus* exceeds the interspecific variation purportedly diagnostic for the three *P. phoebus* group species (*P. phoebus* (F.) *P. smintheus*, and *P. behrii* Edw.). Subsequent work using multiple independent molecular markers supports the recognition of *P. smintheus* and *P. phoebus* (Omoto et al. 2006, Schoville & Roderick 2009), but the reciprocal monophyly of *P. smintheus* and *P. behrii* is not supported, i.e., the genetic variation within *P. smintheus* as currently defined exceeds the variation between *P. smintheus* and *P. behrii* (Schoville & Roderick 2009), suggesting that the species status of *P. behrii* needs to be re-evaluated. We do argue that care must be taken when examining egg microsculpture because significant variation can be attributed to maternal effects. A mechanism accounting for this variation has yet to be found, but it does not appear to be associated with gross female size or the size of eggs.

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