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Color-pattern Modifications and Speciation in Butterflies of the Genus *Vanessa* and its Related Genera *Cynthia* and *Bassaris*

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ABSTRACT—We have previously shown that the systemic injection of sodium tungstate, a protein-tyrosine phosphatase (PTPase) inhibitor, to pupae immediately after pupation efficiently produces characteristic color-pattern modifications on the wings of many species of butterflies including *Vanessa indica* and *Cynthia cardui*. In these species, the black spots reduced in size in response to the treatment. Similar modifications are occasionally seen in the field-caught aberrant individuals. Exceptionally, however, a *C. cardui* individual with enlarged black spots (“reversed” modification pattern) has been reported. Here we show that these modified patterns of *V. indica* and *C. cardui* are quite similar to the normal color-patterns of other *Vanessa* species. *V. indica* with tungstate-induced modifications resembled *V. tameamea*, *V. samani*, and *Bassaris itea*, whereas *V. dilecta*, *V. atalanta*, and *V. dejeanii* are similar to the “reversed” individual. Most features seen in the experimentally-modified *V. indica* were observed throughout the fore- and hindwings of *V. samani*. In contrast, the experimentally-induced color-patterns of *C. cardui* did not parallel variation of *Cynthia* butterflies. Since it has been proposed that a hypothetical transduction pathway with a PTPase for the scale-cell differentiation globally coordinates the wing-wide color-patterns, our findings suggest that spontaneous mutations in genes in this hypothetical pathway might have played a major role in creating new color-patterns and species in the *Vanessa* genus but not in the *Cynthia* genus. This evolutionary mechanism may probably be shared more widely in Lepidoptera, although this would not be a sole determinant for the color-pattern development and evolution.

Key words: *Vanessa indica*, *Cynthia cardui*, color-pattern modification, speciation, sodium tungstate

INTRODUCTION

Lepidopteran wing color-patterns are very diverse. This is largely because the color patterns on the wing surfaces are conspicuous traits for natural selection by predators (Brakefield, 1987; Uesugi, 1991; Grant *et al.*, 1996; Brakefield and Liebert, 2000; Kapan, 2001) and mates (Jiggins *et al.*, 2001; Sweeney *et al.*, 2003). Butterfly wings thus offer an excellent system to investigate from the viewpoint of evolutionary and developmental biology how these various morphogenic traits have been invented and maintained in nature (Nijhout, 1991; Brakefield *et al.*, 1996; Brunetti *et al.*, 2001; Beldade and Brakefield, 2002).

In addition to various natural forms of butterflies from around the world, many aberrant forms of butterflies caught in the field have been reported in lepidopterology journals.

Some of these “spontaneous” aberrant color-patterns have been systematically reviewed in the light of wing homeosis (Sibatani, 1980, 1983a, 1983b). Recent advances in molecular genetics and developmental biology of the fruit fly made it possible to understand, to some extent, the molecular events leading to these aberrant forms (Warren *et al.*, 1994). However, some other aberrant types of butterflies remain to be examined systematically.

Since more than a century ago, many researchers such as Standfuss in Germany and Merrifield in England have tried to produce aberrant forms by artificial means (Sakaguchi, 1981). Temperature shock, for instance, at the late larval and early pupal stages can produce characteristic wing-pattern modifications with fuzzy pattern boundaries in many species of butterflies and moths (Sakaguchi, 1981). More systematic studies on the temperature-shock-induced modifications of the butterfly wing color-patterns have been performed by Nijhout (1984, 1985a) using *Cynthia cardui*, *Cynthia virginianensis*, and *Junonia (Precis) coenia*, all of which belong to the subfamily Nymphalinae of the family

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Nymphalidae. In these species, the induced aberrant patterns can be arranged in a linear progressive series according to the degrees of modification (Nijhout, 1984, 1985a). These modified individuals are considered to be “phenocopies” that are equivalent to *Drosophila* ones (Nijhout, 1984, 1985a, 1991).

Following this work, we have previously shown that using *C. cardui* the “cold-shock property” can be transferred from the cold-shocked to the non-cold-shocked individuals via hemolymph transfusion (Otaki, 1998). This immediately suggests the existence of a “cold-shock hormone” that coordinates many pattern elements throughout wings and further suggests the existence of chemical substances that can mimic the activity of this putative hormone when injected into pupae (Otaki, 1998).

Accordingly, we have demonstrated that the systemic injection of some oxyanions, especially sodium tungstate, can induce similar color-pattern modifications to those induced by the cold-shock treatment in *C. cardui* (Otaki, 1998, 2003). Tungstate injections into *C. cardui* induced small pattern modifications in 100% and extreme modifications in 63% of the treated individuals (Otaki, 1998), whereas the optimized cold-shock treatment induced small pattern modifications in about 60% and extreme modifications only in 2.6% of the treated individuals (Nijhout, 1984). Thus, it is reasonable to speculate that tungstate mimics the action of the putative cold-shock hormone. Furthermore, we demonstrated that tungstate could act on several species of butterflies from different families including a closely-related species, *V. indica*, although induced modification patterns were species-specific (Otaki and Yamamoto, 2004). Since tungstate is a well-known competitive inhibitor for protein-tyrosine phosphatases (PTPases) at the cellular and animal physiology level (Fillat *et al.*, 1992; Goto *et al.*, 1992; Barbera *et al.*, 1994; Matsumoto, 1994; Haque *et al.*, 1995; Li *et al.*, 1995) due to its structural feature (Stuckey *et al.*, 1994; Egloff *et al.*, 1995; Schubert *et al.*, 1995; Fauman *et al.*, 1996; Yang *et al.*, 1998), the PTPase-mediated developmental signal transduction cascade may be involved in determining the color patterns in many species of butterflies. Evolutionary implication of this hypothetical mechanism has already been pointed out in lycaenid butterflies (Otaki and Yamamoto, 2003).

Here we examined the induced modification patterns in *V. indica* and *C. cardui* in detail in the light of “spontaneous” aberrant patterns of these species as well as normal patterns of other butterflies of the genera *Vanessa*, *Cynthia*, and *Bassaris*. Our findings may contribute to elucidating a developmental mechanism that has been the target of evolutionary change at least in the *Vanessa* genus.

MATERIALS AND METHODS

We referred to Field (1971) for phylogenetic systematics of *Vanessa*, *Cynthia*, and *Bassaris* butterflies in this paper, since molecular phylogenetic relations among these species have not

been available. These three genera were considered to be distinct, although in many papers including ours these three genera are all considered to be the single *Vanessa* genus. There are five species in the genus *Vanessa*: *V. indica*, *V. atalanta*, *V. tameamea*, *V. dejeanii*, and *V. samani* as described in Field (1971). Additionally, a new species, *V. dilecta* (Hanafusa, 1992) was discussed, making the total number of the *Vanessa* species six. We also looked into five subspecies of *V. indica* that have been recognized (Field, 1971). In addition, we considered two and nine species of *Bassaris* and *Cynthia*, respectively (Field, 1971). We focused our attention only on the color-patterns in the light of the tungstate-induced modifications, thus our results do not necessarily recapitulate phylogenetic relations.

Experimental procedures for the color-pattern modifications were described elsewhere (Otaki, 1998; Otaki and Yamamoto, 2004). Briefly, sodium tungstate injections of 1.0 M solution were made at the segmental boundaries of the pupae using a micropipette or microsyringe within 6 hours after pupation. Since we were especially interested in the overall wing-wide color-pattern development, the MD (Modification Degree) scoring system, which considers modifications of several pattern elements and synthesizes them as a MD score, was mainly used in this study (Otaki, 1998, 2003; Otaki and Yamamoto, 2004). MD was assigned from 0 (normal) to 5 (most aberrant) as real numbers because modification variations can be aligned in a progressive linear series (Nijhout, 1984; Otaki, 1998; Otaki and Yamamoto, 2004). For the color-pattern analysis of each pattern element, we used terminology of Nijhout (1991) and Scott (1986) together with our conventional terms (Fig. 1).

Modified global color-patterns were quantitatively analyzed based on the following numerals: induction rate (IR), modification-inducing index (MI), and survival rate (SR), which have been described elsewhere (Otaki, 1998; Otaki and Yamamoto, 2004). Briefly, these numerals were calculated as follows:

$$IR(\%) = \frac{n_m}{N_e} \times 100$$

$$MI(\%) = \left[\frac{\sum_{MD=0}^5 n_{MD} MD}{5N_e} \right] \times 100$$

$$SR(\%) = \frac{N_e}{N_t} \times 100$$

where N_e =the number of treated individuals with successful eclosion, and n_m =the number of modified individuals, MD =the modification degrees ranging from 0 to 5, and n_{MD} =the number of individuals modified by the treatment with a defined modification degree, and N_t =the total number of treated individuals.

As a highly prominent feature of wild-type and experimentally-modified *Vanessa* and its related species, relative area of orange (RAO) on the dorsal forewings was expressed as the percentage of orange area relative to an entire wing surface. Area measurements were performed based on the number of pixels in digital images of wings. RAO for each species was then expressed as mean \pm SD. These data were subjected to independent *t*-test to yield separate *p*-values. Statistical analyses were performed using SYSTAT 5.1.2 (SYSTAT, Inc.) and graphically expressed using Excel (Microsoft Corporation).

Published records of naturally occurring aberrant individuals were searched for with the help of Chouken Press, Ltd. (Osaka, Japan) using a comprehensive data collection of all the available professional and amateur reports on Japanese butterflies. Specimens of several *Vanessa*, *Cynthia*, and *Bassaris* species were obtained from dealers and amateur lepidopterists.

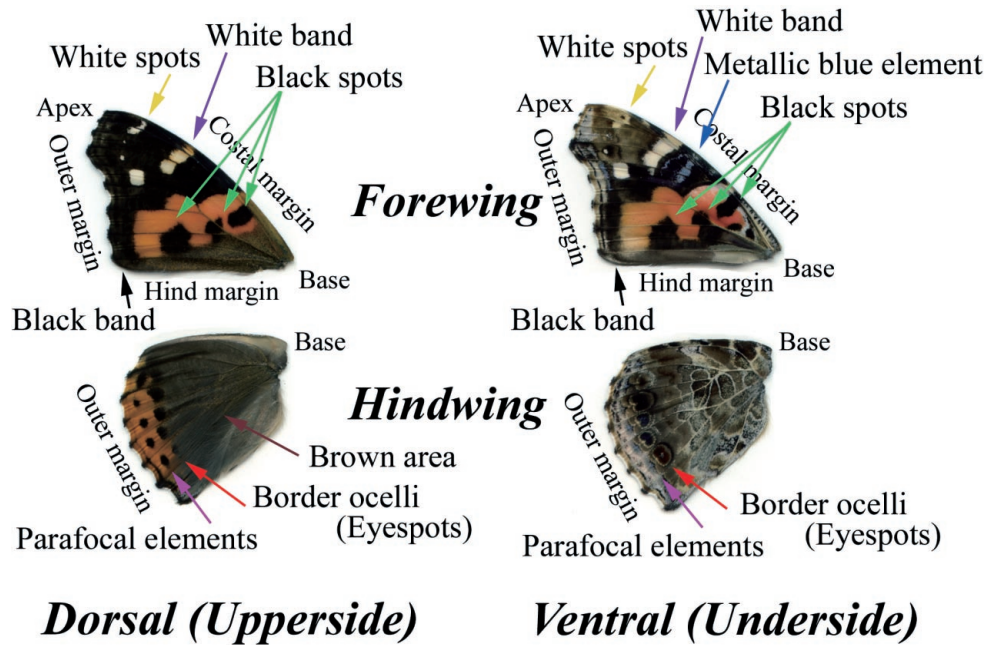


Fig. 1. Terminology for color-pattern elements of *Vanessa*, *Cynthia*, and *Bassaritis* butterflies used in this paper. Here a normal (wild-type) *V. indica* is shown as a representative figure.

RESULTS AND DISCUSSION

Analysis of the experimentally-modified color-patterns of *V. indica* and *C. cardui*

We first examined the tungstate-induced modification patterns of two species, *V. indica* (Otaki and Yamamoto,

2004) and *C. cardui* (Otaki, 1998) (Fig. 2A, B). In these species, overall modification patterns were almost identical to each other. Modified individuals could be arranged in a sequential linear series from MD1 to MD5 (Otaki, 1998; Otaki and Yamamoto, 2004). Among three indicators for the treatment response (Table 1), survival rate (SR) was almost

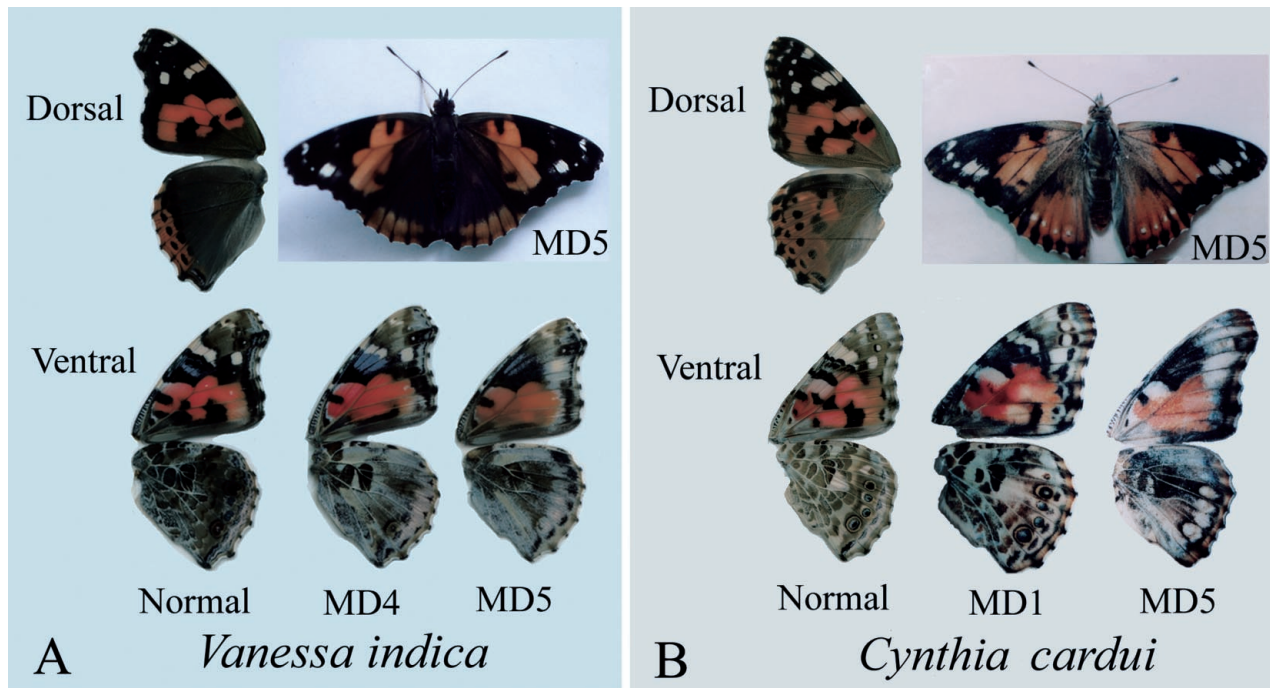


Fig. 2. Tungstate-induced modifications of *V. indica* and *C. cardui*. (A) *V. indica*. (B) *C. cardui*. Modification patterns are quite similar to each other. Live individuals with the most aberrant color-patterns (MD5) are shown at the top right-hand corners. Partly reproduced and modified from Otaki (1998) and Otaki and Yamamoto (2004).

Table 1. Color-pattern modifications of *Vanessa* and *Cynthia* butterflies

Species	Induction rate	Modification-inducing index	Survival rate
<i>Vanessa indica</i> *	69	38	89
<i>Cynthia cardui</i> †	100	76	90
<i>Cynthia virginensis</i> ‡	10	10	unknown

* Reported in Otaki and Yamamoto (2004).

† Reported in Otaki (1998).

‡ Reported in Nijhout (1984) and theoretically estimated in Otaki and Yamamoto (2004).

identical in two species. Induction rate (IR) and modification-inducing index (MI) indicated that *C. cardui* was more easily affected than *V. indica*. Nonetheless, MD5 individuals of *V. indica* were still easily obtained. In contrast, it has been reported that *C. virginensis* was relatively resistant to the treatment. This difference of MI and IR among these species possibly indicates the degrees of canalization of the color patterns.

The most conspicuous response pattern to the treatment was the enlargement of the orange area on forewings and dorsal hindwings (Fig. 2A, B). On the forewings, the black spots (green arrows in Fig. 1) in the orange area successively diminished toward MD5, in which only two spots, one in the submedian area at the costal margin and one in the postmedian area at the hind margin, still existed on the dorsal side. But on the ventral side, the latter black spots also disappeared. Furthermore, the black band (black arrow in Fig. 1) along the outer margin enlarged in width on the dorsal forewings.

The orange area enlarged also on the dorsal hindwings. The parafoveal elements (an array of black spots along the outer margin; pink arrow in Fig. 1) successively “moved” toward the wing base, resulting in the merger with the border ocelli (focal elements; red arrow in Fig. 1) and eventually with the wide brown area (brown arrow in Fig. 1), around which pale orange hue appeared. The border ocelli also seemed to be located somewhat closer to the wing base, and both parafoveal and focal elements reduced in size in the dorsal hindwings in both species. In *C. cardui*, enlargement of the orange area tended to “erase” the normal parafoveal elements, border ocelli, and black spots in the middle of the dorsal hindwings. The border ocelli eventually became white. These white border ocelli were reminiscent of the white spots on the dorsal forewings near the apex (yellow arrow in Fig. 1). This analogy may be reasonable, considering that fore- and hindwings are thought to be morphologically homologous to each other in butterflies (Nijhout, 1991).

On the ventral forewings of *V. indica*, the metallic blue element (blue arrow in Fig. 1) elongated along the costal margin together with the background black area, erasing the white band (purple arrow in Fig. 1) just above it. In *C. cardui*, its corresponding black area also enlarged as in *V. indica*. Interestingly, other white spots (yellow arrow in Fig. 1) around the apex enlarged in both species, which was in sharp contrast to the white band located in the proximity to

the metallic blue element. This fact nicely illustrates the independence of pattern elements even on the same wing surface, as reported in other systems (Nijhout, 1985b; Nijhout and Wray, 1988; Nijhout, 1991; Paulsen and Nijhout, 1993). Enlargement of the black area around the metallic blue spot and enlargement of white spots around the apex appeared to “push in” the array of the white spots located proximity to the metallic blue element from both sides of them, resulting in their shrinkage. On the ventral side of the forewings, enlargement of the white area located along the outer margin blurred the black area. It seemed that there was a distinctive boundary in the middle of the wing that confers cells on the wings different sensitivity to the treatment. This boundary is obviously different from the “M₃ boundary” (Nijhout, 1991) that is known to occur in many species of butterflies. Instead, this seemed to be a boundary of two patterning systems, the central symmetry system and the border ocelli system.

On the ventral hindwings of both species, the parafoveal elements (pink arrow in Fig. 1) “moved” toward the eyespots as in the dorsal sides. Their distinctive eyespot pattern was blurred, resulting in overall white “eyespot”. These white “eyespot” were more conspicuous in *C. cardui*, partly because of the overall darkness of the surrounding area. This again supports the morphological analogy between the white spots in the normal forewings and the eyespots on the normal hindwings.

Complex patterns on the ventral hindwings of both species lost contrast and eventually disappeared, although some pattern elements near the wing base were more resistant than others to the treatment. Likewise, pattern elements at the marginal lines throughout wings were relatively less affected to the treatment, although they also seemed to slightly elongate toward the wing base. Overall wing shapes were never affected at all by the treatment in both species.

Naturally-occurring aberrant color-patterns of *V. indica* and *C. cardui*

We found that some field-caught aberrant individuals showed color patterns that were quite similar, if not identical, to that of the experimentally-induced ones in both species (Matsushita, 1989; Arata, 1994; Iwata, 1995; Fig. 3A–H). This is not totally unexpected, considering that similar modification patterns have been obtained with cold-shock treatments (Nijhout, 1984; Otaki, 1998, 2003). Natural “cold

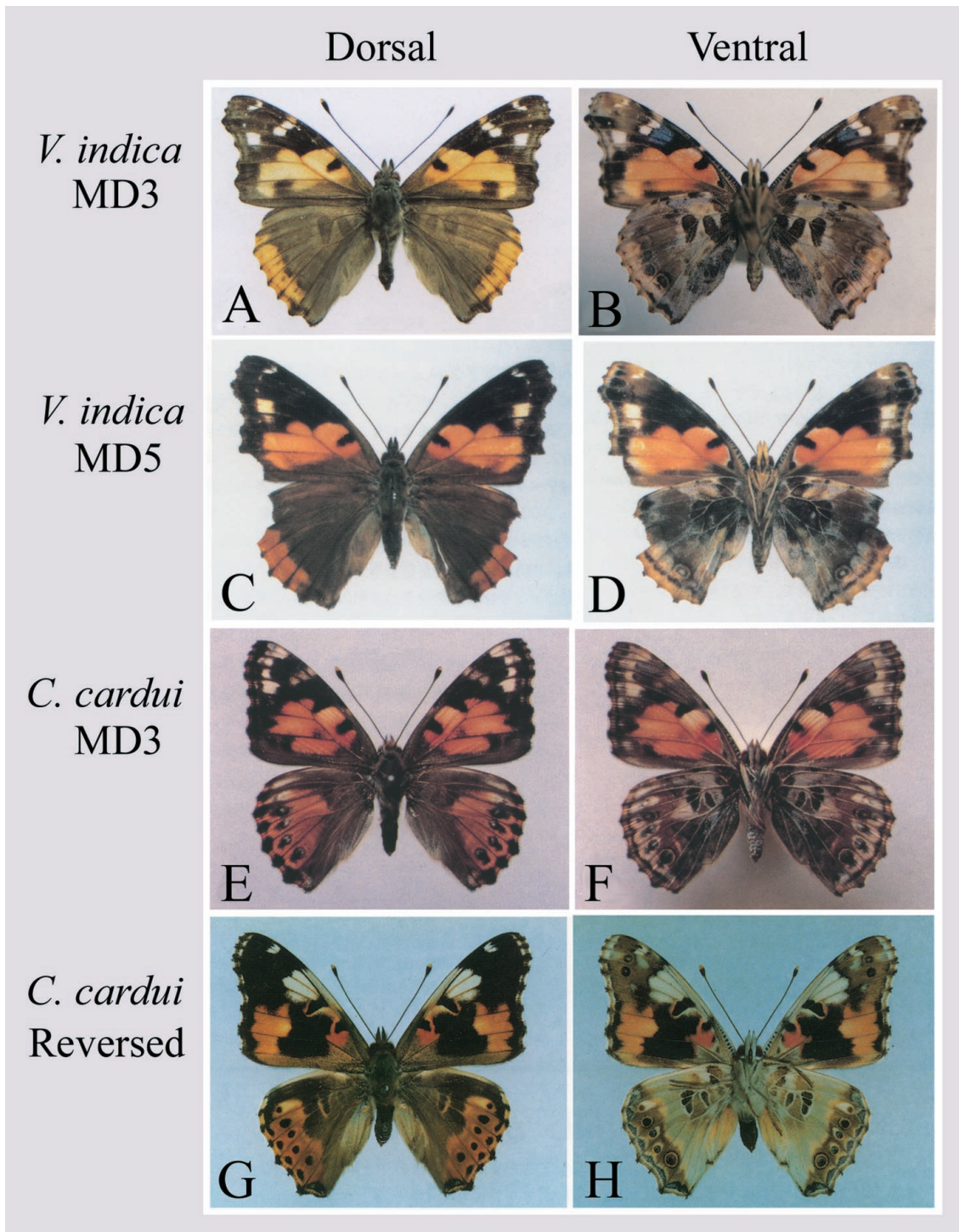


Fig. 3. Field-caught aberrant individuals of *V. indica* and *C. cardui*. (A–D) *V. indica* (Matsushita, 1989; Arata, 1994). (E–H) *C. cardui* (Iwata, 1995; Sumitani, 1988). In both species, modified individuals have occasionally been reported. Especially noteworthy is the “reversed” individual (G, H). This is undoubtedly an extremely rare aberrant individual in the field. Reproduced from *Chouken Filed*.

shock” might have created these aberrant individuals. However, genetic contributions to the naturally-occurring modifications cannot be ignored entirely. Different individuals respond differently to the tungstate or cold-shock treatments in terms of modification degrees, which can be attributed to different genetic background (Nijhout, 1984; Otaki, 1998,

2003; Otaki and Yamamoto, 2004).

Unexpectedly, we found one reported individual of *C. cardui* with the “reversed” aberrant color-patterns (Sumitani, 1988; Fig. 3G, H). In this individual, the black spots on the forewings and also on the dorsal hindwings enlarged in size considerably (instead of reducing as in the experimental

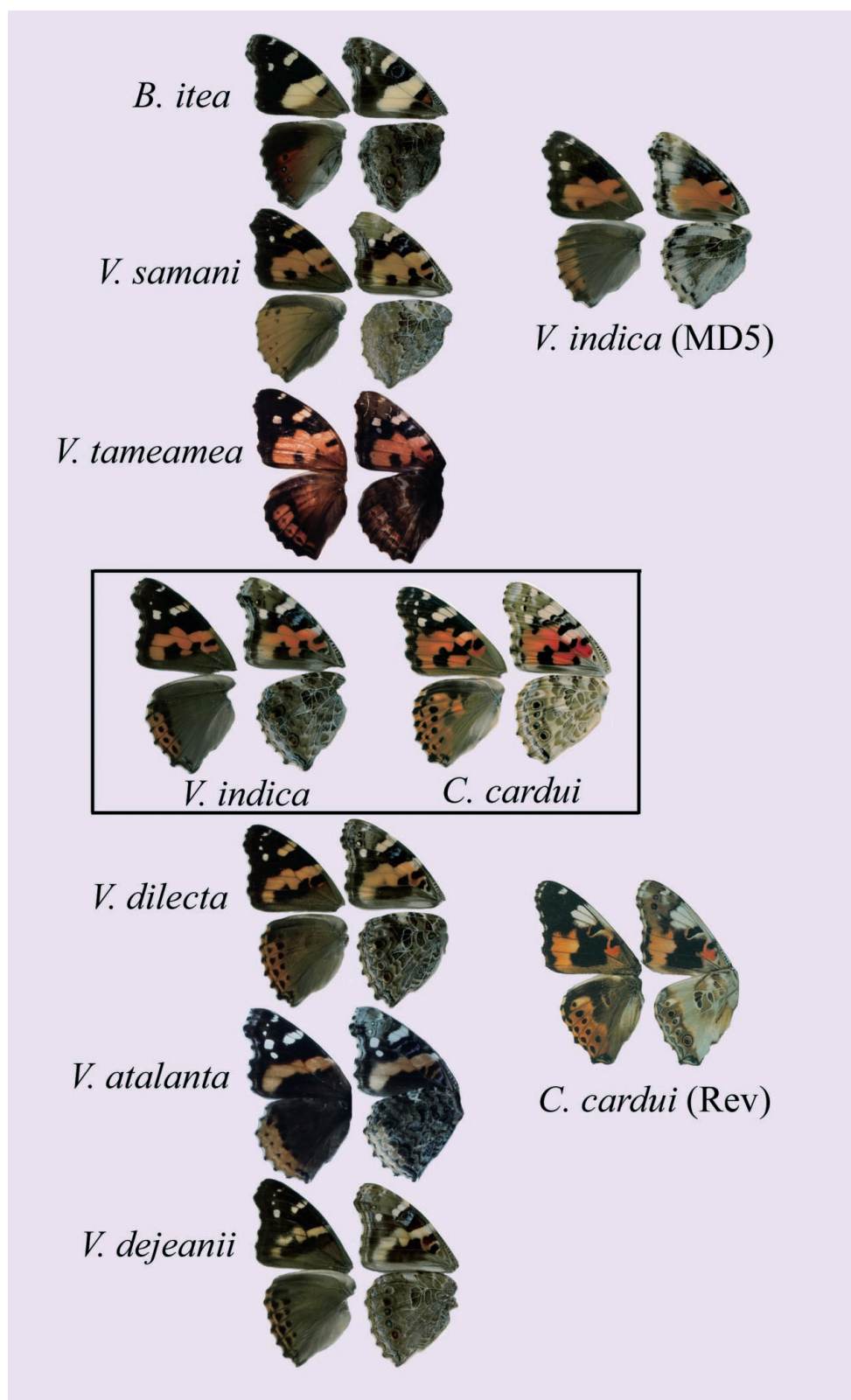


Fig. 4. Comparison of the color patterns of normal (wild-type) *Vanessa* and *Bassaritis* species and those of aberrant *V. indica* and *C. cardui*. In reference to the modified color-patterns (shown at the right side), six *Vanessa* species and one *Bassaritis* species are placed vertically in a linear series of their color-pattern difference (shown at the left side). *V. indica* and *C. cardui* were considered as “prototypes” of speciation in these genera. Wings of *C. cardui* (Rev) were reproduced from Sumitani (1988). Other butterfly wings shown in this figure came from specimens maintained by ourselves.

modifications). Also on the forewings, the white band at the costal margin also enlarged in size (instead of reducing), whereas most white spots near the apex disappeared (instead of enlarging). On the ventral hindwings, the overall color became lighter (instead of darker), and the parafoveal elements and border ocelli moved away from, instead of toward, the wing base. Pattern elements at the marginal lines were much narrower (instead of wider) than those of the normal *C. cardui*. All of these features clearly observed in this individual are in an opposite direction of the experimentally-induced modifications, supporting the notion that it was an individual with “reversed” modification pattern. Although we have not succeeded in producing this type of individuals experimentally, a modification mechanism for this aberrant individual would be fundamentally identical to that of the experimentally-induced one with an opposite “polarity” of hypothetical molecular events. It is worth pointing out that similar phenomena were observed in *Lycaena phlaeas daimio* (Otaki and Yamamoto, 2004).

Comparative analysis of the normal color-patterns of *Vanessa*, *Cynthia*, and *Bassaritis* butterflies

We reasoned that these modifications might have played a role in speciation of *Vanessa* butterflies. We here show that the modification patterns of *V. indica* and *C. cardui* discussed above are quite similar to the normal color-

patterns of other *Vanessa* species (Fig. 4). The *V. indica* individuals with the tungstate-induced modifications resembles *V. tameamea* and *V. samani*, where the black spots on the forewings and the parafoveal and focal elements on the hindwings are smaller in size than the normal *V. indica*. As a result, the orange area occupies broader area in these two species. The parafoveal and focal elements on the ventral hindwings appear to be located somewhat closer to the wing base, although this feature was not very obvious. Also in these species, the black band along the outer margin is wider especially on the ventral side. In *V. samani*, pattern elements in the distal side of the ventral hindwings are blurred overall as if treated with tungstate.

In *Bassaritis itea*, the black spots in the middle of the forewings entirely “disappeared” except one at the costal margin. This spot corresponds to the one most resistant to the tungstate treatment in *V. indica* and *V. cardui*. Also in *B. itea*, the black band along the outer margin is wider than that of other *Vanessa* species, “invading” much to the pale yellow area. Although *B. itea* may not formally be included in the genus *Vanessa* according to Field (1971), these features directly corresponded to the experimentally-induced modifications in *V. indica* and *C. cardui*. On the other hand, the white spots in the apex of *B. itea* are as large as those of the normal *V. indica*, although the metallic blue elements in *B. itea* seem to be “deformed” to some extent. Also, *B.*

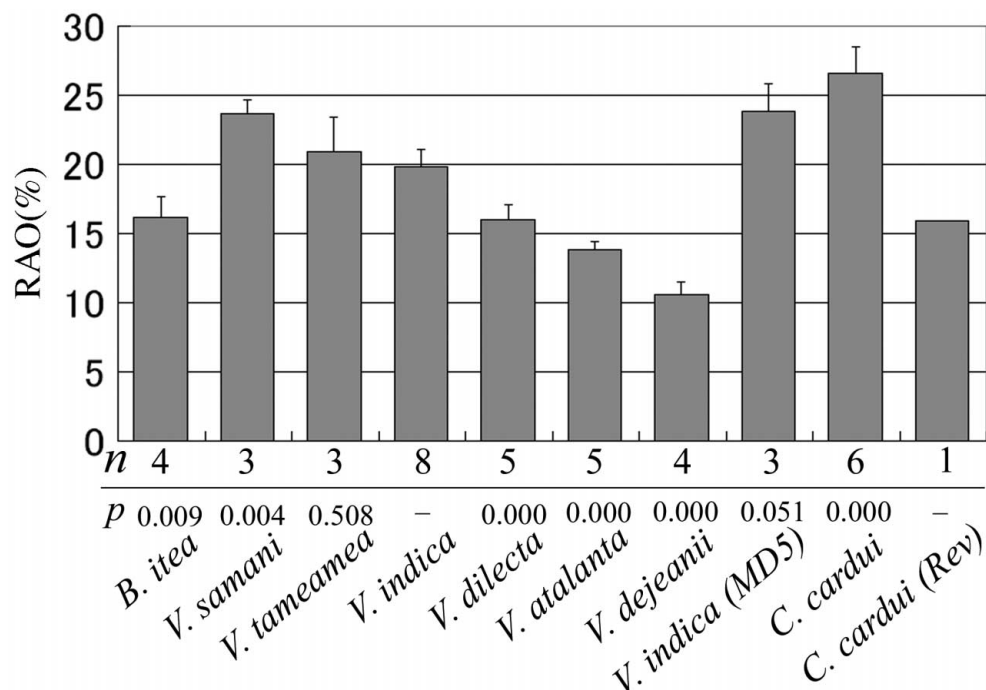


Fig. 5. Relative area of orange (RAO) of normal (wild-type) and aberrant *Vanessa* and other butterflies. The number of individuals examined is indicated at the bottom of each bar expressed as mean±SD. Separate *p*-values derived from independent *t*-test between *V. indica* and other species are also indicated. The linear arrangement of these species (shown in Fig. 4) can be supported quantitatively, although differences among these species in terms of the RAO value are not always highly significant due to variations within a species. The RAO value for *B. itea* is small, but this fact do not contradict its position in Fig. 4, because it has a wide black band invading the pale-yellow area, which is a tendency observed in experimentally-induced modifications of *V. indica*. The MD5 individuals of *V. indica* are different from the normal (wild-type) ones in terms of the RAO value at the level of *p*=0.051. Similarly, the reversed individual of *C. cardui* has very small RAO value compared to that of the normal (wild-type) *C. cardui*.

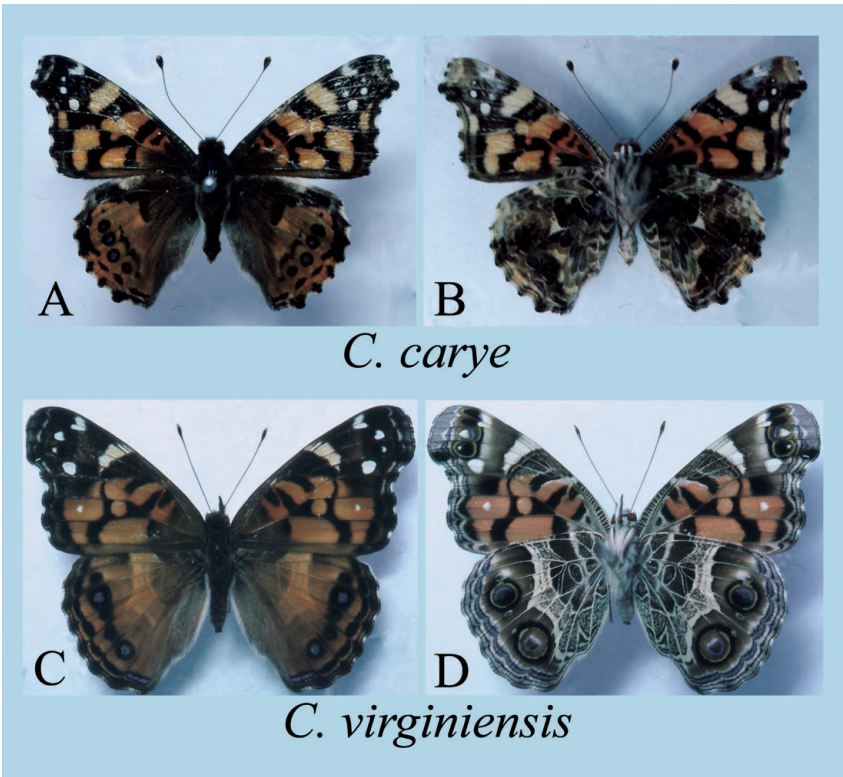


Fig. 6. Two *Cynthia* species. (A, B) *C. carye*. This species shows the color pattern quite similar to *C. cardui*. Other *Cynthia* species such as *C. kershawi* are also similar. (C, D) *C. virginensis*. This species shows the color pattern similar to *C. cardui* except that there are two extremely large eyespots and other very small spots on the hindwings. Specimens of *C. carye* and *V. virginensis* were kindly provided by K. Shiraiwa.

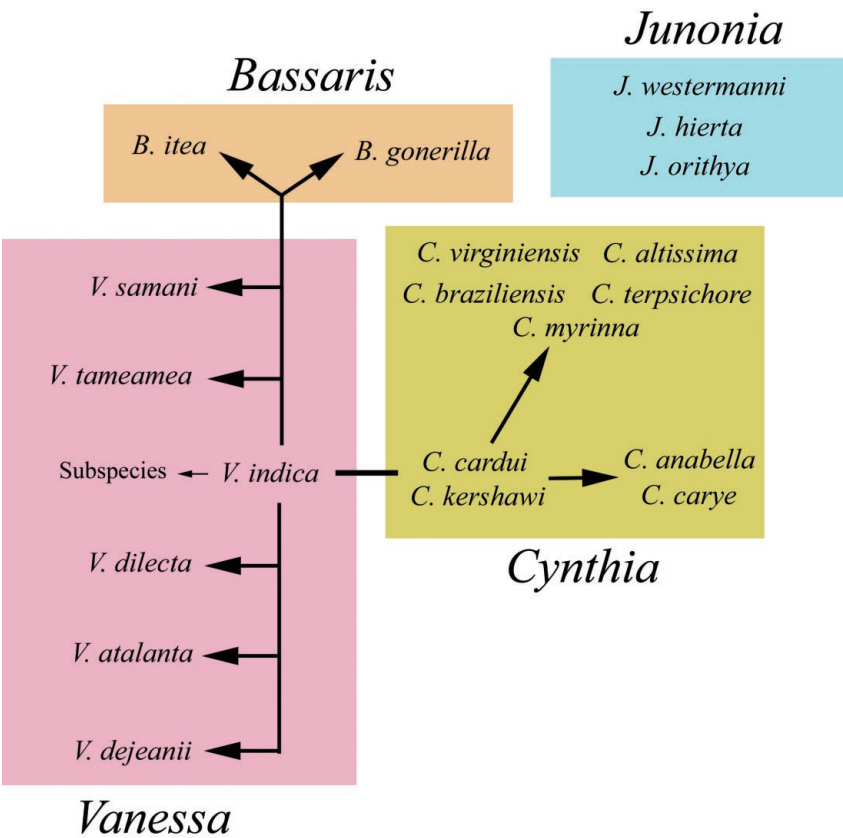


Fig. 7. Possible evolutionary relationship in the genera *Vanessa*, *Cynthia*, *Bassaritis*, and *Junonia* in terms of wing color-patterns.

itea has the overall pattern of the ventral hindwings quite similar to the normal *V. indica* individual. Thus, it appears that in *B. itea*, some specific, but not all, elements were similar to the corresponding ones in the experimentally-induced *V. indica*, suggesting that other evolutionary factors are required for speciation of *Bassaritis*.

In contrast to two *Vanessa* species and one *Bassaritis* species discussed above, other three *Vanessa* species, *V. dilecta* (Hanafusa, 1992), *V. atalanta*, and *V. dejeanii* were similar to the “reversed” individual of *C. cardui*. The black spots in *V. indica* in the middle of the forewings progressively “enlarge” and “merge” with one another in a linear series of *V. dilecta*, *V. atalanta*, and *V. dejeanii*. In *V. dejeanii* and probably also in *V. dilecta*, the parafoveal elements on the hindwings appear to be located somewhat closer to the outer margin than those of the normal *V. indica* individuals, although we cannot exclude the possibility that the parafoveal elements are just bigger and not located differently in these species. The orange color is paler overall in these three species. All of these features were observed in the reversed individual of *C. cardui*. On the other hand, the white bands and the white spots did not seem to be considerably different from those of the normal *V. indica* individual.

This way, we here placed six *Vanessa* species plus one *Bassaritis* species in a linear series of color-pattern differences, which was expressed as a vertical arrangement in Fig. 4. The qualitative analysis above was quantitatively supported by comparing relative area of orange (RAO) of each species, a key phenotypic feature of evolutionary and experimentally-induced modifications (Fig. 5). Among the *Vanessa* genus, the RAO value was largest in *V. samani* and smallest in *V. dejeanii*. Between these two species, it sequentially varied as in the linear arrangement in Fig. 4, although individual variations within a given species existed. Therefore, evolution of *Vanessa* species can be hypothesized to have involved, at least in part, modifications in the PTPase signal transduction pathway.

Whereas all *Vanessa* species can be understood in the framework of the hypothetical signal transduction pathway with some minor divergence, no *Cynthia* species can fit into such a framework despite that *C. cardui* is slightly more easily modified by the tungstate treatment than *V. indica* (Table 1), suggesting that the *Cynthia* genus has different evolutionary history. Three *Cynthia* species, *C. cardui*, *C. kershawi*, and *C. carye* (Fig. 6A, B) are quite similar to each other with several small eyespots on the hindwings, while other *Cynthia* species, *C. virginensis* (Fig. 6C, D), *C. altissima*, *C. brazilensis*, *C. terpsichore*, and *C. myrinna* are known to have two exceptionally large and other smaller eyespots on the hindwings. This trend has never been observed in the *Vanessa* and *Bassaritis* genera. This “enlargement” of the specific eyespots and the “shrinkage” of others may be mainly due to an evolutionary change of either organizing signals or an interpreting molecular system or both, considering available data on other butterfly species (Monteiro *et al.*, 1994, 2003; Beldade *et al.*, 2002; Beldade

and Brakefield, 2003; Breuker and Brakefield, 2003). Intriguingly, this feature seen in *Cynthia* is known to occur in butterflies of the closely-related genus *Junonia* (*Precis*) such as *J. orithya*. Some *Junonia* species such as *J. westermanni* and *J. hierta* also show a feature seen in *Bassaritis*: they lack most black spots in the forewings seen in *Vanessa*. These morphological similarities suggest a close evolutionary relation between the two genera, *Cynthia* and *Junonia*. Possible evolutionary relations among *Vanessa*, *Cynthia*, *Bassaritis*, and *Junonia* were summarized in Fig. 7. This is largely consistent with a recent morphological and molecular analysis of *Vanessa* and other butterflies at the level of genus (Wahlberg and Nylin, 2003), although more detailed molecular analysis at the level of species has not been performed yet.

In conclusion, it is reasonable to speculate that the hypothetical signal transduction pathway played a major role in the diversification of the color-patterns and speciation in *Vanessa* butterflies but not in *Cynthia* butterflies. *Bassaritis* butterflies might have diversified from *Vanessa* as an extension of this evolutionary trend. Since similar evolutionary mechanism is likely to be operating in Lycaenidae as well (Otaki and Yamamoto, 2003), the hypothetical mechanism for the color-pattern determination may be shared by these two families and possibly more widely in Lepidoptera as implicated by the existence of the “Nymphalid Groundplan” (Nijhout, 1991). However, it is also obvious that this mechanism would not be a sole determinant for the color-pattern development and evolution.

This speculation encourages us to identify a target molecule for the putative “cold-shock hormone” and tungstate which might be responsible for the global coordination of the butterfly wing color-patterns and speciation of *Vanessa* butterflies. Since several genes in the epidermal growth factor receptor (EGFR) signaling pathway are known to control growth, patterning, and subdivision of the *Drosophila* wing imaginal disc (Zecca and Struhl, 2002a, b), sequence comparison of these genes from *Vanessa*, *Cynthia*, and *Bassaritis* may clarify evolutionary relations among species of these genera. Furthermore, mutations in these genes in *V. indica* could produce several phenotypes similar to other *Vanessa* species, which will support our findings presented in this paper.

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