INTRODUCTION

In evolutionary developmental biology, how patterning evolves is one of the major questions. The color pattern is easy to perceive visually, and we can trace evolutionary shifts or diversification of color patterns from their phenotypes. Moreover, because color patterns are developed in two dimensions, their measurement and analysis are straightforward and highly reproducible. Fruit flies of the genus Drosophila and their relatives (drosophilids) have been used as model systems, using which we can compare evolutionary gains of traits and parallel evolution. Various groups of drosophilids, including genus Idiomyia (Hawaiian Drosophila), have a variety of pigmentations, ranging from simple black pigmentations around crossveins to a single antero-distal spot and a more complex mottled pattern. Pigmentation patterns are sometimes obviously used for sexual displays; however, in some cases they may have other functions. The process of wing formation in Drosophila, the general mechanism of pigmentation formation, and the transport of substances necessary for pigmentation, including melanin precursors, through wing veins are summarized here. Lastly, the evolution of the expression of genes regulating pigmentation patterns, the role of cis-regulatory regions, and the conditions required for the evolutionary emergence of pigmentation patterns are discussed. Future prospects for research on the evolution of wing pigmentation pattern formation in drosophilids are presented, particularly from the point of view of how they compare with other studies of the evolution of new traits.

KEYWORDS

color pattern, Drosophila biarmipes, Drosophila guttifera, fruit fly, gene regulatory network
the mechanisms of pigmentation pattern formation among diverse species. Studies on various pigmentation patterns and powerful developmental genetic technologies have provided perspectives for elucidating mechanisms by which organismal phenotypic diversity is generated. The purpose of this review is to outline research on the evolution of *Drosophila* pigmentation patterns, including their diversity and physiological regulation, and the roles of cis-regulatory evolution.

## 2 | PIGMENTATION PATTERN DIVERSITY OF DROSOPHILIDS

Over 4,000 species in 72 genera have been described in family Drosophilidae, order Diptera (Toda, 2020; Yassin, 2013). There are two subfamilies, Drosophilinae and Steganinae, the former of which is larger and more extensively studied, and both of which have species with various wing pigmentation patterns. Among the subfamily Drosophilinae, the largest but paraphyletic genus *Drosophila* includes more than 1,160 species (O’Grady & DeSalle, 2018a; Toda, 2020; Figure 1). Many species of Drosophilinae have patterns on their wings (Figure 2), although the most extensively used species in biological studies, *Drosophila melanogaster*, has no such wing pigmentation pattern (Figure 2a). In many species, pigmentation patterns are often related to veins, and with a few exceptions, vein patterns are fairly similar among species (Figure 2b).

Among the Drosophilidae flies with wing pigmentation patterns, the most famous are the groups found in Hawaii. There are two major clades of drosophilids in Hawaii, genus *Idiomyia* (a group that is treated as a subgenus within genus *Drosophila* by some researchers, while some other researchers treat it as unspecified rank “Hawaiian Drosophila”), which is unique to Hawaii, and genus *Scaptomyza*, which includes members that are distributed in Hawaii. Notably, *Idiomyia* includes large-sized species with various wing pigmentation patterns (Edwards, Doescher, Kaneshiro, & Yamamoto, 2007; O’Grady & DeSalle, 2018b; Figure 2c). Studies of Hawaiian drosophilids have greatly contributed to our understanding of speciation and adaptive radiation (Carson, Hardy, Spieth, & Stone, 1970; Carson & Kaneshiro, 1976; Hardy, 1965; O’Grady & DeSalle, 2018b). There is an ongoing debate about how the drosophilids (genus *Idiomyia* and genus *Scaptomyza*) immigrated to Hawaii; especially, there is debate about the number of immigration events from continents (Katoh, Izumitani, Yamashita, & Watada, 2017; Lapoint, O’Grady, & Whiteman, 2013).

Non-Hawaiian drosophilids also include a large number of species with wing pigmentation (Patterson, 1943; Werner, Steenwinkel, & Jaenike, 2018; Wittkopp, Carroll, & Kopp, 2003), and some distinct groups of such species are briefly described below. A group endemic to the Samoan Islands, genus *Samoaiia*, includes species with entirely

---

FIGURE 1  The phylogenetic relationships of representative genera, subgenera and species groups in subfamily Drosophilinae. The genus *Drosophila* is a paraphyletic group, and thus contains many other genera in the clade. Gray letters indicate subgenera and species groups that belong to the genus *Drosophila*. Wing illustrations indicate representative (but not all) pigmentation patterns of each operational taxonomic unit. The tree topology is based on Yassin (2013), except for the treatment of *Drosophila guttifera* (the rightmost illustration in the *quinaria* group), which is included in the *quinaria* group in this figure. The white circle (A) corresponds to the timepoint estimated as 62.9 ± 12.4 million years ago (Tamura, Subramanian, & Kumar, 2004).
The function of animal color patterns have not been clearly elucidated in general, and in particular, the functions of wing pigmentation in drosophilids have not been clearly elucidated. Males of the *suzukii, elegans* and *rhopaloa* subgroups (*melanogaster* group, subgenus *Sophophora*) with sex-specific antero-distal wing pigments extend their wings and display them to conspecific females, suggesting that pigmentation contributes to their mating success (Hegde, Chethan, & Krishna, 2005; Massey et al., 2019; Revadi et al., 2015; Setoguchi et al., 2014). The effect of pigmentation on mating success is not always strong, and how strongly pigmentation contributes to this success varies among studies (Fuyama, 1979; Roy & Gleason, 2019; Singh & Chatterjee, 1987; Yeh, Liou, & True, 2006). Hawaiian *Idiomyia* includes species with sexually dimorphic and monomorphic species, and species in both of these categories perform wing display during their courtships (Spieth, 1966).

Some non-Hawaiian drosophilids have sexually monomorphic pigmentation, whose function has not been well studied. These pigments might have various functions, such as crypsis, aposematism or thermoregulation, but none of these possibilities have been well examined. In two species of Tephritids (Tephritidae, Diptera), wing pigmentation patterns were suggested to have the function of mimicry of jumping spiders, to repel other predatory jumping spiders (Greene, Orsak, & Whitman, 1987; Mather & Roitberg, 1987).
Shevtsova et al. found that some membranous wings of flies and wasps have wing interference patterns, which are particular types of structural color. These are colorful interference patterns in weakly pigmented membranous regions without black or brown color, and they are visible when the background is dark. The authors suggested the possibility that they function in visual communication, such as mating display, taking Drosophila as an example (Shevtsova, Hansson, Janzen, & Kjærandsen, 2011). Further studies will be needed to clarify the functions of color patterns and visual systems of drosophilids.

4 | HOW DOES PIGMENTATION OCCUR?
PHYSIOLOGICAL ASPECTS

The process of wing formation has been well studied in D. melanogaster (Blair, 2007; Johnson & Milner, 1987). The area within a wing disc called the “pouch”, which is formed in the larva, extends and forms a bag-like pupal wing that consists of two layers of epithelia. The epithelial cells proliferate, become folded, and secrete cuticles to prepare an adult wing. The wing extends after eclosion, forming the adult wing of full size. The epithelial cells detach from the cuticle, undergo apoptosis and are redirected to the body trunk by hemolymph flow (Kimura, Kodama, Hayashi, & Ohta, 2004; Link et al., 2007). These processes of wing formation are considered to be conserved among drosophilids.

The mechanisms of melanin biosynthesis and accumulation in the cuticle have been well studied using the abdominal epidermis of D. melanogaster (Gibert, Mouchel-Vielh, & Peronnet, 2017), and the mechanisms in the wings are believed to be largely the same as those in the abdomen. One of the most famous body color mutations in D. melanogaster is the yellow mutant (Morgan & Bridges, 1916), which is now known to be due to an abnormality in the gene encoding the Yellow protein involved in melanin synthesis, although the precise molecular function of this protein is still unknown.

One hypothesis proposes that Yellow is an enzyme involved in melanin synthesis (Wittkopp, True, & Carroll, 2002). Another hypothesis is that Yellow protein acts like a hormone or growth factor, an idea based on the fact that yellow mutation acts non-cell-autonomously for pigmentation and also affects male mating success, which is reminiscent of the actions of vertebrate pigmentation-related hormones, such as α-melanocyte-stimulating hormone and adrenocorticotropic hormone (Drapeau, 2003). The predicted enzymatic activity of Yellow as Dopa chrome converting enzyme (DCE) was not detected, and instead proteins belonging to the same protein family, Yellow-f and Yellow-f2, were found to have DCE activity (Han et al., 2002). The protein coding sequence of yellow suggested the presence of a signal peptide in the N terminus, and Yellow protein was observed to be extracellularly localized (Geyer, Spana, & Corces, 1986; Wittkopp et al., 2002). Taking all this evidence together, the hypothesis that appears most likely to be correct is that the Yellow protein serves as an anchoring pigment in the cuticle layer (Geyer et al., 1986; Hinaux et al., 2018).

The ebony gene has been identified as the causative gene of a mutant with darker body color. The Ebony protein enzymatically converts dopamine to N-β-alanyl dopamine (NBAD), and it thereby suppresses black pigmentation (Wittkopp et al., 2002; Wright, 1987). The tan gene has been identified from a mutation in body color, and Tan protein has been shown to enzymatically convert NBAD into dopamine, the reverse reaction to that of Ebony protein (True et al., 2005). Concerning possible functions in the pigmentation process and inter-specific differences, these three genes (yellow, ebony and tan) are particularly well-studied “effector genes” (Jeong et al., 2008; Rebeiz, Pool, Kassner, Aquadro, & Carroll, 2009). Regarding regulation of pigmentation in the abdomen, male-specific pigmentation was shown to be controlled positively by Abdominal-B and negatively by bric à brac genes, and pigmentation common to both sexes is positively controlled by optomotor-blind (Kopp, Duncan, Godt, & Carroll, 2000). Many other genes, such as abdominal-A, homothorax, and pou domain motif 3, are also known to affect body pigmentation (Dembeck, Huang, Carbone, & Mackay, 2015; Kalay et al., 2016; Kopp, 2009; Massey & Wittkopp, 2016; Mummery-Widmer et al., 2009; Rogers et al., 2014; Yassin et al., 2016), and those genes might have functions in wings.

The black and brown pigmentation of the Drosophila wing is thought to be caused by the deposition of melanin pigment in the cuticle layer, as in the abdomen. True, Edwards, Yamamoto, and Carroll (1999) examined how pigmentation gene mutations and overexpression alter wing color of Drosophila melanogaster. Also, transport of melanin precursors (especially dopamine) through veins has been suggested to have important roles in pigmentation, based on experimental evidence from wing incubations in medium and wing vein amputations of D. biarmipes and Idiomia grimshawi. Fukutomi, Matsumoto, Agata, Funayama, and Koshikawa (2017) showed that wing pigmentation of D. guttifera and Drosophila biarmipes started in stage P11 (among the stages of pupal development defined as P1–P15; Fukutomi, Matsumoto, Funayama, & Koshikawa, 2018), and that the pigmentation process continued even after the epithelial cells detached from the wing and were retrieved by the body trunk in young adults. The importance of hemolymph circulation in veins was shown by vein amputation, a finding that was similar to that from a previous study by True et al. (1999).

5 | THE LARGE PIGMENTATION PATCH IN THE ANTERO-DISTAL PART OF THE WING: THE MECHANISM OF PIGMENTATION PATTERN IN D. biarmipes

Drosophila biarmipes is a frequently used model species of wing pigmentation formation. It belongs to the suzukii subgroup (melanogaster group, subgenus Sophophora), and typical of the species in this subgroup, it has male-specific antero-distal pigmentation in its wings. True et al. (1999) showed that addition of DOPA or dopamine can enhance pigmentation development in adult wings just after eclosion. Wittkopp et al. (2002) showed that Yellow protein
was localized in the area of pigmentation, and Ebony protein was localized in the area lacking pigmentation, in pupal wings, as indicated by antibody staining.

Gompel, Prud'homme, Wittkopp, Kassner, and Carroll (2005) examined the enhancer activities of the cis-regulatory region around the yellow gene of D. biarmipes. A DNA fragment of D. biarmipes, which was homologous to an enhancer that weakly drives yellow throughout the wing in D. melanogaster, was introduced into D. melanogaster together with EGFP gene to visualize transcriptional activity. Expression was strongly driven at the antero-distal part of the wing, the region of D. biarmipes pigmentation. This suggested that the ancestral weak enhancer was elaborated in the lineage of D. biarmipes, in which it gained strong activity in the antero-distal part of the wing. The absence of yellow expression in the posterior region was explained by repression by Engrailed through its binding to the enhancer region. Distal-less was identified as a positive regulator of yellow by RNAi screening (Arnoult et al., 2013). In pupal wings of D. biarmipes, Distal-less was expressed in the antero-distal region. Ectopic expression of Distal-less upregulated yellow (as visualized through enhancer activity) and downregulated ebony, and RNAi of Distal-less caused downregulation of yellow and upregulation of ebony. Based on this evidence, it was concluded that Distal-less regulates melanin synthesis through regulation of effector genes (Figure 3). Comparisons of these findings with those in related species led to the suggestion that the evolution of the Distal-less expression pattern has contributed to the diversification of the pigmentation pattern.

Because the wingless gene was known to be expressed in the wing outer margin and around crossveins in D. melanogaster, it was the first candidate of the putative trans factor giving input to the vein spot CRE. The wingless gene was expressed in centers of prospective pigmentation spots, and ectopic expression of wingless along longitudinal veins induced ectopic pigmentation, which supported the conclusion that wingless is the factor inducing pigmentation in wings (Figure 3; Werner et al., 2010). wingless is a homolog of vertebrate Wnt-1, and encodes a signal ligand that is secreted from cells and transduces the signal to adjacent cells (Swarup & Verheyen, 2012). Because Wingless proteins were known to diffuse or be transported to a distance of only a few cell diameters in D. melanogaster (with the distance differing depending on the context and the particular study), it was thought that Wingless diffused from the source cells to certain areas and induced pigmentation there (Werner et al., 2010).

**6 | POLKA-DOT PATTERN WITH MANY BLACK SPOTS: MECHANISM OF PIGMENTATION PATTERN FORMATION OF D. guttifera**

D. guttifera belongs to the quinaria group (or to a group very closely related to the quinaria group) of subgenus Drosophila. It has black pigmentation around the crossveins, longitudinal vein tips and campaniform sensilla, making a polka-dot pattern throughout the wings (Koshikawa, Matsumoto, & Fukutomi, 2017). The Yellow protein was localized in the places of prospective pigmentation spots of pupal wings (Gompel et al., 2005). Werner, Koshikawa, Williams, and Carroll (2010) observed that yellow mRNA was expressed in the polka-dot pattern in pupal wings. The enhancer which drove the polka-dot pattern (vein spot CRE) was identified within the cis-regulatory region of the yellow gene using a transgenic reporter assay. The vein spot CRE that originated from D. guttifera, however, drove the expression in the wing outer margin and around crossveins when introduced to a different host species, D. melanogaster. This suggests that the trans environment is different between D. guttifera and D. melanogaster.

**7 | THE MECHANISM OF COLOR PATTERN EVOLUTION: ROLES OF CIS-REGULATORY CHANGE AND CONSTRUCTION OF REGULATORY NETWORK**

The expression pattern of wingless in D. guttifera was unique compared to its pattern in other species of the quinaria group, and
therefore, this pattern was assumed to have evolved after the divergence of *D. guttifera* from other species. When they searched for enhancers around wingless, Koshikawa et al. (2015) found three different enhancer activities unique to *D. guttifera*. One drove expression in longitudinal vein tips, another did so in campaniform sensilla on wings, and the other did so in thoracic stripes. Gains of these enhancer activities were considered to be responsible for the evolution of new expression domains of wingless in *D. guttifera*. (Koshikawa, 2015; Koshikawa et al., 2015). Comparison between *D. guttifera* and *D. melanogaster* revealed that the function of the cis-regulatory region of yellow had also diverged evolutionarily (Werner et al., 2010).

This inter-specific comparison revealed the cis-regulatory evolution of at least two genes, the upstream regulatory gene wingless and the downstream effector gene yellow. Prud'homme et al. (2006) compared the cis-regulatory regions of yellow of *D. elegans* (*elegans* subgroup, subgenus Sophophora) and *D. tristis* (*obscura* group, subgenus Sophophora), and found that enhancers that drove antero-distal pigmentations in wings had evolved independently in different species. In *D. biarmipes*, the function of the yellow regulatory region had evolved (Gompel et al., 2005), and the expression pattern of its regulator Distal-less had also evolved in comparison with that in *D. melanogaster* (Arnoult et al., 2013). Thus, it was revealed that there were complex molecular levels of evolution behind the relatively simple-looking *Drosophila* wing pigmentations. This series of studies provided examples not only of the evolution of wing pigmentation patterns, but of the evolution of gene expression patterns and biological traits by cis-regulatory changes (Carroll, 2005, 2008).

### 8 | FUTURE DIRECTION 1: HOW DID PIGMENTATION DIVERSITY OF DROSOPHILA EMERGE?

It is now clear that the *Drosophila* pigmentation patterns are very diverse, but not much is known about the mechanisms that generate the diversity. First, how many effector genes, such as genes encoding enzymes, are required for pigmentation formation is unknown. Gompel et al. (2005) observed that a *D. melanogaster* wing with overexpression of the yellow gene in an ebony mutant background showed “slight darkening”, but not intense pigmentation. Riedel, Vorkel, and Eaton (2011) also showed that almost no pigmentation was induced by yellow overexpression in wings. These effects were different from that observed on the abdomen, where yellow overexpression induced visible pigmentation and the combination of this overexpression with mutation of *ebony* resulted in obvious black pigmentation (Wittkopp et al., 2002). Thus, multiple effector genes may need to work in concert to produce pigmentation patterns in wings.

Fukutomi, Kondo, Toyoda, Shigenobu and Koshikawa (2020) screened genes specifically expressed in pigmentation areas of pupal wings of *D. guttifera*. Analysis of a combination of multiple transcriptome data sets enabled identification of a set of genes expressed in the pigmentation area and also regulated by wingless, and this set is expected to include all of the genes required for pigmentation and expression at this developmental stage. As mentioned in the above section, in addition to genes expressed in wings, precursor(s) of melanin and/or signaling molecules transported through veins might also contribute to the inter-specific differences of pigmentation.

### 9 | FUTURE DIRECTION 2: HOW DO NEW TRAITS EMERGE?

Regarding the evolution of color patterns, there have been a great number of reports in recent years about how butterfly (*Rhopalocera, Lepidoptera*) color patterns are controlled and have evolved (Monteiro, 2015). It is now technically possible to identify genes that control color patterns using genomics, and to investigate gene functions by RNAi and genome editing (Kunte et al., 2014; Nishikawa et al., 2015; Zhang & Reed, 2016). Both in *Drosophila* and in butterflies, the yellow family and melanin synthesis genes contribute to pigmentation formation (Zhang, Martin, et al., 2017). Notably, some of the regulatory genes for color pattern, such as Distal-less and Wnt ligand genes, have been shown to act both in *Drosophila* and in butterflies (Arnoult et al., 2013; Connahs et al., 2019; Martin et al., 2012; Martin & Reed, 2014; Mazo-Vargas et al., 2017; Werner et al., 2010; Zhang & Reed, 2016).

On the other hand, it remains unknown whether or not some genes reported to regulate color patterns in butterflies, such as optix and cortex (Nadeau et al., 2016; Reed et al., 2011; Zhang, Mazo-Vargas, & Reed, 2017b), have such functions in drosophilids. Based on the fact that drosophilids and butterflies are phylogenetically distant, and they have pigmentation on non-homologous parts of the wings, i.e., that drosophilids have pigmentation on the membranous part of wings while butterflies mostly have pigmentation on scales, the mechanisms of color pattern formation are considered to have evolved independently in these two groups. Comparison of these independent systems would be fruitful for finding general rules of color pattern formation.

In addition, a number of studies revealed mechanisms of color pattern formation and pigmentation in other insect systems, such as caterpillars (larvae of butterflies), moths, and ladybeetles, and comparisons with them would be valuable (Ando et al., 2018; Futahashi, Banno, & Fujiwara, 2010; Gautier et al., 2018; Suzuki, Koshikawa, Vargas, & Reed, 2017b), have such functions in drosophilids. Based on the fact that drosophilids and butterflies are phylogenetically distant, and they have pigmentation on non-homologous parts of the wings, the mechanisms of color pattern formation are considered to have evolved independently in these two groups. Comparison of these independent systems would be fruitful for finding general rules of color pattern formation.

Vertebrates have pigment cells specified for pattern formation and pigmentation in other insect systems, such as caterpillars (larvae of butterflies), moths, and ladybeetles, and comparisons with them would be valuable (Ando et al., 2018; Futahashi, Banno, & Fujiwara, 2010; Gautier et al., 2018; Suzuki, Koshikawa, Vargas, & Reed, 2017b) have such functions in drosophilids. Based on the fact that drosophilids and butterflies are phylogenetically distant, and they have pigmentation on non-homologous parts of the wings, the mechanisms of color pattern formation are considered to have evolved independently in these two groups. Comparison of these independent systems would be fruitful for finding general rules of color pattern formation.

Relative to *D. melanogaster*, both *D. biarmipes* and *D. guttifera* seem to have experienced evolution of multiple genes, including...
the downstream yellow gene. How the gene expression of multiple genes has evolved in concert during the process of evolution of one particular trait would be a good point of view from which to explore the evolution of novel traits. In particular, some critical questions are how existing gene regulatory networks that specify pre-patterns and networks of effector genes required for pigmentations acquire new connections, and how many genetic mutations are required for acquiring such connections (Prud’homme, Gompel, & Carroll, 2007; Rebeiz & Tsiantis, 2017). Recently, in addition to the various color patterns discussed above, the genetic basis of evolution of new traits and character states, such as trichome patterns of Drosophila larvae, Drosophila adult terminaria, early development and skeletogenesis of sea urchins, beetle horns, and fans of water striders, have been studied (Gao & Davidson, 2008; Glassford et al., 2015; Moczek & Rose, 2009; Rebeiz & Williams, 2017; Santos, Le Bouquin, Crumière, & Khila, 2017; Stern & Carroll, 2005). Chance caught on the wing: Cis-regulatory evolution and the origin of pigment patterns in Drosophila. Nature, 433, 481–487. https://doi.org/10.1038/nature03235

ACKNOWLEDGEMENTS
I thank Cédric Finet for a wing image, Yuichi Fukutomi for comments, and Elizabeth Nakajima for English editing. A part of this work was supported by KAKENHI (17K19427, 18H02486) and Yamada Science Foundation.

REFERENCES
Ando, T., Matsuda, T., Goto, K., Hara, K., Ito, A., Hirata, J., ... Niimi, T. (2018). Repeated inversions within a pannier intron drive diversification of intraspecific colour patterns of ladybird beetles. Nature Communications, 9, 3843. https://doi.org/10.1038/s41467-018-06116-1

Arnoult, L., Su, K. F., Manoel, D., Minervino, C., Magriña, J., Gompel, N., & Prud’homme, B. (2013). Emergence and diversification of fly pigmentation through evolution of a gene regulatory module. Science, 339, 1423–1426. https://doi.org/10.1126/science.1233749

Blair, S. S. (2007). Wing vein patterning in Drosophila and the analysis of intercellular signaling. Annual Review of Cell and Developmental Biology, 23, 293–319. https://doi.org/10.1146/annurev.cellbio.23.090506.123606

Carroll, S. B. (2005). Evolution at two levels: On genes and form. PLoS Biology, 3, e245. https://doi.org/10.1371/journal.pbio.0030245

Carroll, S. B. (2008). Evo-devo and an expanding evolutionary synthesis: A genetic theory of morphological evolution. Cell, 134, 25–36. https://doi.org/10.1016/j.cell.2008.06.030

Carson, H. L., Hardy, D. E., Spieth, H. T., & Stone, W. S. (1970). The evolutionary biology of the Hawaiian Drosophilidae. In M. K. Hecht, & W. C. Steere (Eds.), Essays in evolution and genetics in honor of Theodosius Dobzhansky (pp. 437–543). New York, NY: Springer (Meredith Corporation). https://doi.org/10.1002/0471-9781-1461-9585-4.15

Carson, H. L., & Kaneshiro, K. Y. (1976). Drosophila of Hawaii: Systematics and ecological genetics. Annual Review of Ecology and Systematics, 7, 311–345. https://doi.org/10.1146/annurev.es.07.110176.001523

Chialvo, C. H. S., White, B. E., Reed, L. K., & Dyer, K. A. (2019). A phylogenetic examination of host use evolution in the quinaria and testacea groups of Drosophila. Molecular Phylogenetics and Evolution, 130, 233–243. https://doi.org/10.1016/j.ympev.2018.10.027

Connahs, H., Tili, S., van Creij, J., Loo, T. Y., Banerjee, T. D., Saunders, T. E., & Monteiro, A. (2019). Activation of butterfly eye spots by Distalless is consistent with a reaction-diffusion process. Development, 146, dev169367. https://doi.org/10.1242/dev.169367

Dembeck, L. M., Huang, W., Carbone, M. A., & Mackay, T. F. (2015). Genetic basis of natural variation in body pigmentation in Drosophila melanogaster. Fly (Austin), 9, 75–81. https://doi.org/10.1080/1933934.2015.1102807

Drapeau, M. D. (2003). A novel hypothesis on the biochemical role of the Drosophila Yellow protein. Biochemical and Biophysical Research Communications, 311, 1–3. https://doi.org/10.1016/j.bbrc.2003.09.106

Edwards, K. A., Doescher, L. T., Kaneshiro, K. Y., & Yamamoto, D. (2007). A database of wing diversity in the Hawaiian Drosophila. PLoS ONE, 2, https://doi.org/10.1371/journal.pone.0000487

Fukutomi, Y., Kondo, S., Toyoda, A., Shigenobu, S., & Koshikawa, S. (2020). Transcriptome analysis reveals wingless regulates neural development and signaling genes in the region of wing pigmentation of a polka-dotted fruit fly. bioRxiv, https://doi.org/10.1101/2020.01.09.899864

Fukutomi, Y., Matsumoto, K., Agata, K., Funayama, N., & Koshikawa, S. (2017). Pupal development and pigmentation process of a polka-dotted fruit fly, Drosophila guttata (Insecta, Diptera). Development Genes and Evolution, 227, 171–180. https://doi.org/10.1007/s00404-017-0578-3

Fukutomi, Y., Matsumoto, K., Funayama, N., & Koshikawa, S. (2018). Methods for staging pupal periods and measurement of wing pigmentation of Drosophila guttata. Journal of Visualized Experiments, 131, e56935. https://doi.org/10.3791/56935

Futahashi, R., Banno, Y., & Fujihara, H. (2010). Caterpillar color patterns are determined by a two-phase melanin gene prepatternning process: New evidence from tan and laccase2. Evolution and Development, 12, 157–167. https://doi.org/10.1111/j.1525-142X.2010.00401.x

Fuyama, Y. (1979). A visual stimulus in the courtship of Drosophila suzukii. Experientia, 35, 1327–1328. https://doi.org/10.1007/BF01963987

Gao, F., & Davidson, E. H. (2008). Transfer of a large gene regulatory apparatus to a new developmental address in echinoid evolution. Proceedings of the National Academy of Sciences of the United States of America, 105, 6091–6096. https://doi.org/10.1073/pnas.0801201105

Gautier, M., Yamaguchi, J., Foucaud, J., Loiseau, A., Ausset, A., Facon, B., ... Prud’homme, B. (2018). The genomic basis of color pattern polymorphism in the harlequin ladybird. Current Biology, 28, 3296–3302. e7. https://doi.org/10.1016/j.cub.2018.08.023

Geyer, P. K., Spana, C., & Corces, V. G. (1986). On the molecular mechanism of gypsy-induced mutations at the yellow locus of Drosophila melanogaster. The EMBO Journal, 5, 2657–2662. https://doi.org/10.1002/j.1460-2075.1986.tb04548.x

Gilbert, J. M., Mouchel-Vielh, E., & Peronnet, F. (2017). Modulation of yellow expression contributes to thermal plasticity of female abdominal pigmentation in Drosophila melanogaster. Scientific Reports, 7, 43370. https://doi.org/10.1038/srep43370

Glassford, W. J., Johnson, W. C., Dall, N. R., Smith, S. J., Liu, Y., Boll, W., ... Rebeiz, M. (2015). Co-option of an ancestral Hox-regulated network underlies a recently evolved morphological novelty. Developmental Cell, 34, 520–531. https://doi.org/10.1016/j.devcel.2015.08.005

Gompel, N., Prud’homme, B., Wittkopp, P. J., Kassner, V. A., & Carroll, S. B. (2005). Chance caught on the wing: Cis-regulatory evolution and the origin of pigment patterns in Drosophila. Nature, 433, 481–487. https://doi.org/10.1038/nature03235
Greene, E., Orsak, L. J., & Whitman, D. W. (1987). A tephidit fly mimics the territorial displays of its jumping spider predators. *Science*, 236, 310–312. https://doi.org/10.1126/science.236.4799.310

Grimaldi, D. A. (1987). Phylogenetics and taxonomy of Zygotorica (Diptera: Drosophilidae). *Bulletin of the American Museum Natural History*, 186, 104–268.

Han, Q., Fang, J., Ding, H., Johnson, J. K., Christensen, B. M., & Li, J. (2002). Identification of *Drosophila melanogaster* yellow-f and yellow-F2 proteins as dopachrome-conversion enzymes. *Biochemical Journal*, 368, 333–340. https://doi.org/10.1042/bj20020272

Hardy, E. D. (1965). Diptera: Cyclorrhapha II. Series schizophora, section acalypterae i, family drosophilidae. *Insects of Hawaii* (Vol. 12). Honolulu, HI: University of Hawai‘i Press.

Hegde, S. N., Chethan, B. K., & Krishna, M. S. (2005). Mating success of males with and without wing patch in *Drosophila* biarmipes. *Indian Journal of Experimental Biology*, 43, 902–909.

Hinaux, H., Bachem, K., Battistara, M., Rossi, M., Xin, Y., Jaenichen, R., Kopp, A., & True, J. R. (2002). Evolution of male sexual characters in the origin of sexually dimorphic characters in *Drosophila*. *Evolution & Development*, 4, 277–278. https://doi.org/10.1016/j.evodevo.2000.09.003

Izumitani, H. F., Kusaka, Y., Koshikawa, S., Toda, M. J., & Katoh, T. (2016). Identification of *Drosophila melanogaster* yellow-f and yellow-F2 proteins as dopachrome-conversion enzymes. *Biochemical Journal*, 368, 333–340. https://doi.org/10.1042/bj20020272

Kalay, G., Lusk, R., Dome, M., Hens, K., Deplancke, B., & Wittkopp, P. J. (2016). Potential direct regulators of the *Drosophila yellow* gene identified by yeast one-hybrid and RNAi screens. *G3: Genes Genomes Genet.*, 6, 3419–3430. https://doi.org/10.1534/g3.116.023602

Kato, T., Izuimtani, H. F., Yamashita, S., & Watada, M. (2017). Multiple origins of Hawaiian *drosophilids*: Phylogeography of *Scaptomyza* Hardy (Diptera: *Drosophilidae*). *Entomological Science*, 20, 33–44. https://doi.org/10.1111/ens.12222

Kawahara, S. (2015). Enhancer modularity and the evolution of new traits. *Fly (Austin)*, 9, 155–159. https://doi.org/10.1080/19339346.2015.1151129

Koshikawa, S., Giorgianni, M. W., Vaccaro, K., Kassner, V. A., Yoder, J. H., Werner, T., & Carroll, S. B. (2015). Gain of cis-regulatory activities underlies novel domains of wingless gene expression in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 7524–7529. https://doi.org/10.1073/pnas.1509022112

Koshikawa, S., Matsumoto, K., & Fukutomi, Y. (2017). *Drosophila* gut-tierra as a model system for unravelling color pattern formation. In T. Sekimura & H. F. Nijhout (Eds.), *Diversity and evolution of butterfly wing patterns* (pp. 287–301). Singapore: Springer. https://doi.org/10.1007/978-981-10-4956-9_16

Kunze, K., Zhang, W., Tenger-Trolander, A., Palmer, D. H., Martin, A., Reed, R. D., ... Kronforst, M. R. (2014). *doublesex* is a mimicry supergene. *Nature*, 507, 229–232.

Lapoint, R. T., O’Grady, P. M., & Whiteman, N. K. (2013). Diversification and dispersal of the Hawaiian *Drosophilidae*: The evolution of *Scaptomyza*. *Molecular Phylogenetics and Evolution*, 65, 95–108.

Link, N., Chen, P. O., Lu, W. J., Pogue, K., Chuong, A., Mata, M., ... Abrams, J. M. (2007). A collective form of cell death requires homeodomain interacting protein kinase. *Journal of Cell Biology*, 178, 567–574. https://doi.org/10.1083/jcb.200702125

Malloch, J. R. (1934). *Part VI. Diptera. Drosophilidae. Insects of Samoa and other Samoan terrestrial Arthropoda*. British Museum (Natural History). Pp. 267–312.

Markow, T. A., & O’Grady, P. (2006). *Drosophila: A guide to species identification and use*. New York, NY: Academic Press.

Martin, A., Papa, R., Nadeau, N. J., Hill, R. I., Counterman, B. A., Halder, G., ... Reed, R. D. (2012). Diversification of complex butterfly wing patterns by repeated regulatory evolution of a Wnt ligand. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 12632–12637. https://doi.org/10.1073/pnas.1204800109

Martin, A., & Reed, R. D. (2014). Wnt signaling underlies evolution and development of the butterfly wing pattern symmetry systems. *Developmental Biology*, 395, 367–378. https://doi.org/10.1016/j.ydbio.2014.08.031

Massey, J. H., Gavin, R., Rice, G. R., Firdaus, A., Chen, C. Y., Yeh, S. D., Wittkopp, P. J. (2019). Co-evolving wing spots and mating displays are genetically separable traits in *Drosophila*. *bioRxiv*, https://doi.org/10.1101/869016

Massey, J. H., & Wittkopp, P. J. (2016). The genetic basis of pigmentation differences between and within *Drosophila* species. *Current Topics in Developmental Biology*, 119, 27–61. https://doi.org/10.1016/bstcb.2016.03.004

Mather, M. H., & Roitberg, B. D. (1987). A sheep in wolf’s clothing: Tephritid flies mimic spider predators. *Science*, 236, 308–310. https://doi.org/10.1126/science.236.4799.308

Mazo-Vargas, A., Concha, C., Livraghi, L., Massardo, D., Wallbank, R. W. R., Zhang, L., ... Martin, A. (2017). Macroevolutionary shifts of WntA function potentiate butterfly wing-pattern diversity. *Proceedings of the National Academy of Sciences of the United States of America*, 114, 10701–10706.

Moczek, A. P., & Rose, D. J. (2009). Differential recruitment of limb patterning genes during development and diversification of beetle horns. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 8992–8997. https://doi.org/10.1073/pnas.0809668106

Monteiro, A. (2015). Origin, development, and evolution of butterfly eyespots. *Annual Review of Entomology*, 60, 253–271. https://doi.org/10.1146/annurev-ento-010814-020942

Morgan, T. H., & Bridges, C. B. (1916). *Sex-linked inheritance in Drosophila* (p. 237). Washington, DC: Carnegie Institution of Washington Publication.

Mummery-Widmer, J. L., Yamazaki, M., Stoeger, T., Novatchkova, M., Bhalerao, S., Chen, D., ... Knoblich, J. A. (2009). Genome-wide analysis of Notch signalling in *Drosophila* by transgenic RNAi. *Nature*, 458, 987–992. https://doi.org/10.1038/nature07936

Nadeau, N. J., Pardo-Diaz, C., Whibley, A., Supple, M. A., Saenko, S. V., Wallbank, R. W., ... Jiggins, C. D. (2016). The gene cortex controls
mimicry and crypsis in butterflies and moths. Nature, 534, 106–110. https://doi.org/10.1038/nature17961

Nishikawa, H., Iijima, T., Kajitani, R., Yamaguchi, J., Ando, T., Suzuki, Y., ... Fujiwara, H. (2015). A genetic mechanism for female-limited Batesian mimicry in Papilio butterfly. Nature Genetics, 47, 405–409.

O’Grady, P. M., & DeSalle, R. (2018a). Phylogeny of the genus Drosophila. Genetics, 209, 1–25.

O’Grady, P., & DeSalle, R. (2018b). Hawaiian Drosophila as an evolutionary model clade: Days of future past. BioEssays, 40, 1700246. https://doi.org/10.1002/bies.201700246

Okada, T. (1976). Subdivision of the genus Chymomyza Czerny (Diptera, Drosophilidae), with description of three new species. Kontyû, 44, 496–511.

Patterson, J. T. (1943). The Drosophilidae of the Southwest. The University of Texas Publication, 4313, 7–216.

Prud’homme, B., Gompel, N., & Carroll, S. B. (2007). Emerging principles of regulatory evolution. Proceedings of the National Academy of Sciences, 104, 8605–8612. https://doi.org/10.1073/pnas.0700488104

Prud’homme, B., Gompel, N., Rokas, A., Kassner, V. A., Williams, T. M., Yeh, S.-D., ... Carroll, S. B. (2006). Repeated morphological evolution through cis-regulatory changes in a pleiotropic gene. Nature, 440, 1050–1053. https://doi.org/10.1038/nature04597

Rebeiz, M., Pool, J. E., Kassner, V. A., Aquadro, C. F., & Carroll, S. B. (2009). Stepwise modification of a modular enhancer underlies adaptation in a Drosophila population. Science, 326, 1663–1667. https://doi.org/10.1126/science.1178357

Rebeiz, M., & Tsiantis, M. (2017). Enhancer evolution and the origins of morphological novelty. Current Opinion in Genetics & Development, 45, 115–123. https://doi.org/10.1016/j.gde.2017.04.006

Rebeiz, M., & Williams, T. M. (2017). Using Drosophila pigmentation traits to study the mechanisms of cis-regulatory evolution. Current Opinion in Insect Science, 19, 1–7. https://doi.org/10.1016/j.cois.2016.10.002

Reed, R. D., Papa, R., Martin, A., Hines, H. M., Counterman, B. A., Pardo-Díaz, C., ... McMillan, W. O. (2011). optix drives the repeated convergent evolution of butterfly wing pattern mimicry. Science, 333, 1137–1141. https://doi.org/10.1126/science.1208227

Revadi, S., Lebreton, S., Witzgall, P., Anfora, G., Dekker, T., & Becher, P. (2015). Sexual behavior of Drosophila Suzuki. Insects, 6, 183–196. https://doi.org/10.3390/insects6010183

Riedel, F., Verkeld, E., & Eaton, S. (2011). Megalin-dependent Yellow endocytosis restricts melaninization in the Drosophila cuticle. Development, 138, 149–158. https://doi.org/10.1242/dev.056309

Werner, T., Koshikawa, S., Williams, T. M., & Carroll, S. B. (2010). Generation of a novel wing colour pattern by the Wingless morphogen. Nature, 464, 1143–1148. https://doi.org/10.1038/nature08896

Werner, T., Steenwinkel, T., & Jaenike, J. (2018). Drosophilids of the Midwest and Northeast. Version 2. Open Access Books (Vol. 1). Houghton, MI: Michigan Technological University.

Wittkopp, P. J., Carroll, S. B., & Kopp, A. (2003). Evolution in black and white: Genetic control of pigment patterns in Drosophila. Trends in Genetics, 19, 495–504. https://doi.org/10.1016/S0168-9525(03)00194-X

Wittkopp, P. J., True, J. R., & Carroll, S. B. (2002). Reciprocal functions of the Drosophila Yellow and Ebony proteins in the development and evolution of pigment patterns. Development, 129, 1849–1858.

Wright, T. R. (1987). The genetics of biogenic amine metabolism, sclerotization, and melanization in Drosophila melanogaster. Advances in Genetics, 24, 127–222. https://doi.org/10.1016/S0065-2660(08)60008-5

Yamaguchi, J., Banno, Y., Mitā, K., Yamamoto, K., Ando, T., & Fujiwara, H. (2013). Periodic Wnt1 expression in response to ecdysteroid generates twin-spot markings on caterpillars. Nature Communications, 4, 1857. https://doi.org/10.1038/ncomms2778

Yassin, A. (2013). Phylogenetic classification of the Drosophilidae. Rondani (Diptera): The role of morphology in the postgenomic era. Systematic Entomology, 38, 349–364. https://doi.org/10.1111/j.1365-3113.2012.00665.x

Yassin, A., Delaney, E. K., Reddiex, A. J., Seher, T. D., Bastide, H., Appleton, N. C., ... Kopp, A. (2016). The pmd3 locus is a hotspot for recurrent
evolution of female-limited color dimorphism in Drosophila. *Current Biology*, 26, 2412–2422. https://doi.org/10.1016/j.cub.2016.07.016

Yeh, S. D., Liou, S. R., & True, J. R. (2006). Genetics of divergence in male wing pigmentation and courtship behavior between *Drosophila elegans* and *D. gunungcola*. *Heredity*, 96, 383–395. https://doi.org/10.1038/sj.hdy.6800814

Zhang, L., Martin, A., Perry, M. W., van der Burg, K. R., Matsuoka, Y., Monteiro, A., & Reed, R. D. (2017a). Genetic basis of melanin pigmentation in butterfly wings. *Genetics*, 205, 1537–1550.

Zhang, L., Mazo-Vargas, A., & Reed, R. D. (2017b). Single master regulatory gene coordinates the evolution and development of butterfly color and iridescence. *Proceedings of the National Academy of Sciences of the United States of America*, 114, 10707–10712. https://doi.org/10.1073/pnas.1709058114

Zhang, L., & Reed, R. D. (2016). Genome editing in butterflies reveals that *spalt* promotes and *Distal-less* represses eyespot colour patterns. *Nature Communications*, 15, 11769. https://doi.org/10.1038/ncomms11769

**How to cite this article:** Koshikawa S. Evolution of wing pigmentation in *Drosophila*: Diversity, physiological regulation, and cis-regulatory evolution. *Develop Growth Differ*. 2020;62:269–278. https://doi.org/10.1111/dgd.12661