

Gynandromorphs as Indicators of Modularity and Evolvability in Ants

ANDREW S. YANG^{1*} AND EHAB ABOUHEIF²

¹The School of the Art Institute of Chicago, Chicago, Illinois

²McGill University, Montreal, Quebec, Canada



ABSTRACT

Gynandromorphs, individuals that display a mosaic of male and female tissues or cell populations, have been extensively documented in solitary and social insects for over 100 years. Yet the evolutionary significance of gynandromorphs has remained obscure. Here we describe our discovery of a gynandromorph in the hyperdiverse ant genus *Pheidole* whose pattern of bilateral head mosaicism occurs repeatedly across a wide range of ant species. Based on our findings, we propose that gynandromorphs and other mosaic forms may provide new insights into the modularity and evolvability of morphological traits. *J. Exp. Zool. (Mol. Dev. Evol.)* 314B, 2011. © 2011 Wiley-Liss, Inc.

J. Exp. Zool.
(Mol. Dev. Evol.)
314B, 2011

How to cite this article: Yang AS, Abouheif E. 2011. Gynandromorphs as indicators of modularity and evolvability in ants. *J. Exp. Zool. (Mol. Dev. Evol.)* 314B:[page range].

One of the oldest debates in evolutionary biology concerns the question of what types of mutation are relevant to the evolutionary process (Provine, '71; Akam, '98; Stern, 2000; Ronshaugen et al., 2002; Dietrich, 2003; Hoekstra and Coyne, 2007; Carroll, 2008). Older debates have largely centered on the relative importance of continuous vs. discontinuous variation (Provine, '71), as well as the relative importance of micro- vs. macromutations (Dietrich, 2003), especially in developmentally important genes (Akam, '98; Stern, 2000; Ronshaugen et al., 2002). One famous example is Goldschmidt's ('40) highly contentious proposal that large-scale homeotic mutations (which cause one structure to develop where another one normally would) could represent "hopeful monsters" that are the basis for the origin of new species or higher taxa (Dietrich, 2003). More recent debates have focused on the relative importance of mutations that occur in protein coding, in cis-regulatory or in other regions of the genome for generating morphological novelty during evolution (Alonso and Wilkins, 2005; Hoekstra and Coyne, 2007; Wray, 2007; Carroll, 2008).

Here, we focus on gynandromorphs—another yet largely neglected type of mutation—and ask whether they are important to our understanding of evolution. Gynandromorphs are individuals that display a mosaic of distinctly male and female tissues or cell populations. For more than 100 years, these mutations have drawn the attention of both developmental and social insect biologists. For developmental biologists, gynandromorphs have served as a powerful tool to mark cell lineages and compartments (Garcia-Bellido and Merriam, '69). In *Drosophila*,

for example, experimental induction of gynandromorphic tissues played a key role in confirming that chromosomes are the genetic material, because they provide clear phenotypes that correspond to the loss or disruption of sex chromosomes (Morgan and Bridges, '19; Mavor, '24; Patterson, '31). In contrast, social insect biologists have speculated on what gynandromorphs could reveal about the evolutionary and developmental origins of caste determination in social insects. Gynandromorphs and various anomalous "intercaste" or "intersex" mutants were considered to be crucial evidence in extensive mid-century debates over whether the determination of female castes was genetic or environmental (Wheeler, '19, '31, '37; Gregg, '38; Whiting, '38; Morley, '46). Despite this long and rich history of research on gynandromorphs, the potential evolutionary relevance of these mutations has remained largely unexplored. Based on our discovery of a gynandromorph from the hyperdiverse ant genus *Pheidole*, we propose that gynandromorphic mutations help reveal the developmental processes that facilitate the evolution of new phenotypes in social insects.

*Correspondence to: Andrew S. Yang, The School of the Art Institute of Chicago, Room 609, 112 S. Michigan Ave., Chicago, IL, 60603.

E-mail: ayang@saic.edu

Received 29 May 2010; Revised 27 October 2010; Accepted 7 February 2011

Published online in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/jez.b.21407

In 2003, we collected colonies of *Pheidole morrisi* in Long Island, New York, for laboratory observation. We later discovered a gynandromorph working within one of these laboratory colonies. Colonies of almost all *Pheidole* species have a reproductive male caste, and at least three different female castes: a reproductive queen, a sterile worker, and a sterile “soldier” caste. The gynandromorph’s body was that of a soldier, but the left side of the head was that of a male (Fig. 1A and B; confirmed by S. Cover of the MCZ, Harvard University). The male portion of the head is clearly identifiable by the following features: a characteristically larger eye, the presence of an ocellus, male antennal morphology, a reduced mandible, posterior cephalic sculpture, and reduction in cephalic size (Fig. 1A and B). Although we initially considered this mutant as simply an anomaly, we were surprised to find that an almost identical gynandromorph had been described more than 70 years earlier in a different *Pheidole* species (*P. pallidula*) from a different continent (Fig. 1C; Vandel, '31).

The remarkable similarity of these two gynandromorphic mutants in *Pheidole* prompted us to ask whether gynandromorphs in other ant species also exhibit a similar pattern of bilateral mosaicism in the head. In searching the literature, we indeed found a number of disparately related species that show almost the same type of gynandromorphic head mutation as

those described in *P. morrisi* and *P. pallidula* (Fig. 2; including Wheeler, '19, '37; Crawley, '20; Cokendolpher and Francke, '83; Jones and Phillips, '85; Taber and Francke, '86; Kinomura and Yamauchi, '94; Heinze and Trenkle, '97; Yoshizawa et al., 2009). In his book, *Mosaics and Other Anomalies Among Ants*, William M. Wheeler conducted the most comprehensive analysis of gynandromorphs. He was the first to observe the prevalence of mosaicism in ants, particularly within the head (Wheeler, '03). In describing a colony from the genus *Cephalotes*, Wheeler ('37; p 34) wrote:

A problem equally obscure and intriguing is presented by the strict limitation of the mosaic characters to the cephalic region...this limitation, which is characteristic in many of the described gynandromorphs, is most striking in the more than 4,000 cases of these anomalies in *Cephalotes*, because in fully 95 percent of them the male component is confined to the head. The processes responsible for this particular segregation present interesting problems in development and will be discussed at further length in a future paper.

As his book was completed only days before his death, Wheeler never had the chance to further explore the “obscure and intriguing” pattern of cephalic mosaicism. However, researchers

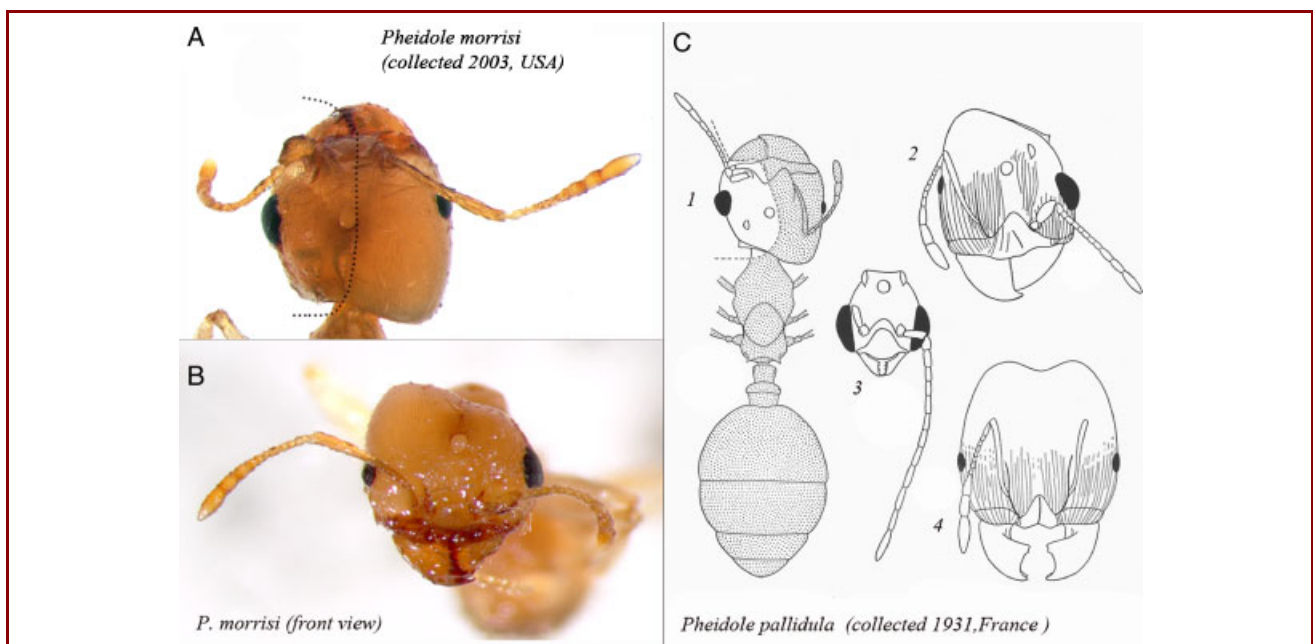


Figure 1. A female soldier-male gynandromorph of *Pheidole morrisi* and *Pheidole pallidula*. (A) Top view of *P. morrisi* female soldier-male gynandromorph. Dotted line showing the area to the left that exhibits the male phenotype in cephalic traits including the eye, ocellus, head shape, mandibles, and antennal configuration. (B) Head-on view of *P. morrisi* female soldier-male gynandromorph. (C) Female soldier-male gynandromorph of *Pheidole pallidula* (1-4), as described by Vandel ('31) and Wheeler ('31, '37); the stippled portions indicates the female soldier phenotype, whereas the clear area denotes the head region exhibiting the male phenotype. Illustrations (3) and (4) show the heads of species-typical males and soldiers, respectively. Image taken from Wheeler ('37).

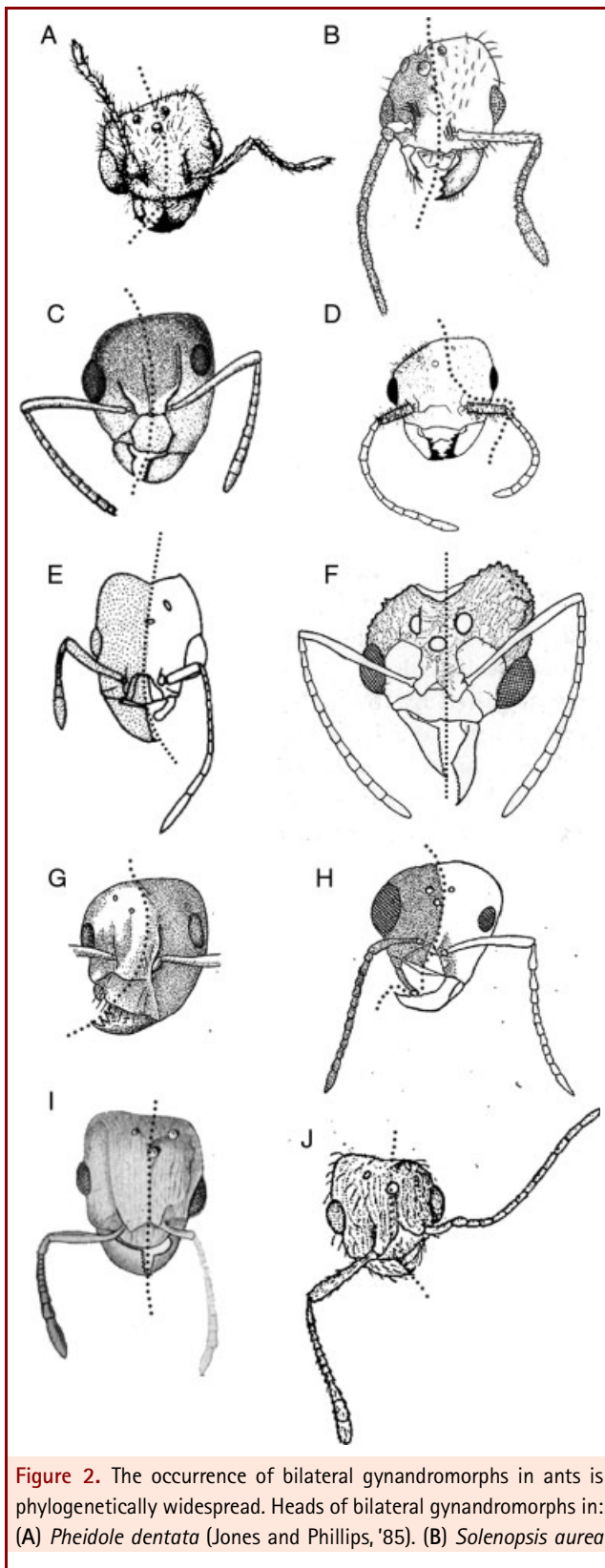


Figure 2. The occurrence of bilateral gynandromorphs in ants is phylogenetically widespread. Heads of bilateral gynandromorphs in: (A) *Pheidole dentata* (Jones and Phillips, '85). (B) *Solenopsis aurea*

have continued to note a similar prevalence of mosaicism limited to the head in ants (Wilson, '84; Jones and Phillips, '85; Yoshizawa et al., 2009). What biological significance might be found in the occurrence of this gynandromorphic pattern?

GYNANDROMORPHS AS INDICATORS OF DEVELOPMENTAL COMPARTMENTS AND MODULES IN THE ANT HEAD

In ants and other insects, sexual dimorphism is often pronounced, making gynandromorphs visually striking and easily detectable. For this reason geneticists studying *Drosophila* have used gynandromorphs to identify developmental "compartments" in the head (Hotta and Benzer, '70; Baker, '78; Haynie and Bryant, '86). Developmental compartments are functional units that correspond to specific morphological structures in the adult and form when adjacent cell populations in a developing tissue can no longer mix with one another, because they are restricted from crossing a cell lineage boundary (Irvine and Rauskolb, 2001). In a similar manner, we propose that bilateral head mosaics (Fig. 2) indicate the existence of a developmental compartment in the head. Within the Hymenoptera, gynandromorphic mutations are thought to occur through a number of mechanisms, including the loss of expression of sex loci in certain cells (Yoshizawa et al., 2009), anomalous fertilization events (Michez et al., 2009), or even inheritance of certain maternal effects (Kamping et al., 2007). Any such mutation within an established compartment during the development of the head will result in a population of cells with a visible and compartmentalized male identity.

Gynandromorphic head mutations, however, are not only limited to the frequently observed bilateral mosaics (Yoshizawa et al., 2009). Any gynandromorphic mutations that occur before the establishment of the developmental compartments of the head will produce variable patterns of mosaic tissue (Fig. 3). This may be explained by the timing of the establishment of compartments in the head relative to other tissues. *Drosophila*, a model holometabolous insect, has served as a successful guide for identifying conserved developmental processes in ants (Abouheif and Wray, 2002; Khila and Abouheif, 2008, 2010; Shbailat et al., 2010). The compartments in most *Drosophila* structures, such as the wing and leg, are established early in development during embryogenesis (Carroll et al., 2005). Unlike these structures, the compartments that give rise to the head are established much later, during larval development (Lawrence and Morata, '76). If this is a conserved feature in ants, then it would explain why



Figure 2. (Continued) (Cokendolpher and Francke, '83). (C) *Camponotus albocinctus* (Wheeler, '31). (D) *Pogonomyrmex* (Taber and Francke, '86). (E) *Cardiocondyla emery* (Heinze and Trenkle, '97). (F) *Acromyrmex octospinosus* (Wheeler, '37). (G) *Polyergus rufescens*. (H) *Camponotus ligniperdus*. (I) *Tetramorium simillimum*. (J) *Epipheidole inquilina* (G–J as reported and redrawn in Wheeler, '03).

mosaicism can occur generally throughout the head and are not limited to the bilateral mosaics we described in Figures 1 and 2. This raises the possibility that the head as a whole represents a “developmental module” (Schlosser and Thieffry, 2000; von Dassow et al., 2000; Gilbert, 2006). We, therefore, hypothesize that the head behaves as a functionally integrated and modular unit that is composed of a collection of coordinated subunits (compartments). The head module thus interacts with—but is separate from—other developmental modules in the body.

Gynandromorphs as Indicators of Evolutionary Modules in the Ant Head

Heads show a remarkable degree of morphological diversity and are thought to have played a key role in the amazing evolutionary and ecological success of ants (Hölldobler and Wilson, '90). Given the apparent developmental modularity and the notable variation in morphology, we propose that the head in ants not only represents a developmental module, but also an “evolutionary module.” As described in many other species, the *P. morrisoni* gynandromorph we discovered seemed to be a functional member of its colony (Wheeler, '37; Donisthorpe, '46; Pearson and Child, '80; Kinomura and Yamauchi, '94; Heinze and Trenkle, '97; Yoshizawa et al., 2009). This observation highlights the fact that while head mosaics indicate developmental instability, they at the same time demonstrate a remarkable tolerance for the anomalies generated by this instability. The ability for individuals with such substantial head mosaicism to be viable and functioning members of their colonies supports the notion that the head is both a developmental and evolutionary module that permits significant variation. A recent morphometric study across hundreds of *Pheidole* species by Pie and Traniello (2007) provides considerable quantitative evidence for the hypothesis that the head is a unique site of morphological variation in ants. They show that variation in head morphologies, including antennal, mandibular, and eye structures, accounted for the greatest amount of variability in the anatomy of these ants (second only to variation in total body size). Pie and Traniello (2007) conclude that there is a high degree of morphological “modularity” between structures in the head relative to the rest of the body that is significant to the evolutionary diversification observed in this particularly species-rich clade.

Wheeler, in his monograph on mosaics ('37), hypothesized that some parasitic ant species and their unique cephalic features might have had their origin in mutations similar to those found in gynandromorphs. Indeed, Wilson ('84) found that within different parasitic species of *Pheidole* the majority of changes occurred in the mandibles, antennae, and related cephalic structures. Furthermore, many of the head mosaics found in gynandromorphs in other species, such as *Vollenhovia emeryi*, correspond with the distinct functional and adaptive traits of the head identified to vary most greatly in *Pheidole*. These traits include (a) the mandible, (b) the mandible+antennae, (c) the head posterior and mouthparts,

or (d) the whole suite of head traits together (Fig. 3; Kinomura and Yamauchi, '94). We believe that such gynandromorphs, like homeotic mutants, are not hopeful monsters. Rather, they represent phenotypic indicators of modularity and evolvability (sensu Wagner and Altenberg, '96) of specific traits in the head.

Future Considerations and Directions

Gynandromorphs are only one type of anomalous and mixed morphological form that exists in ants. Intercastes, for example, are mosaics of different castes of the same sex, such as queens and workers. Intercastes can exhibit a similar kind of bilateral head mosaicism as those of gynandromorphs (Fig. 4), and in ants and bees both gynandromorphs and intercastes can be experimentally generated through temperature shock (Drescher and Rothenbuhler, '63; Berndt and Kremer, '82, '83). This raises the possibility that gynandromorphs and intercastes are produced by similar developmental mechanisms. Therefore, studying mosaic phenotypes more generally will provide a unified approach to examining the evolvable potential of developmental modules, such as the head.

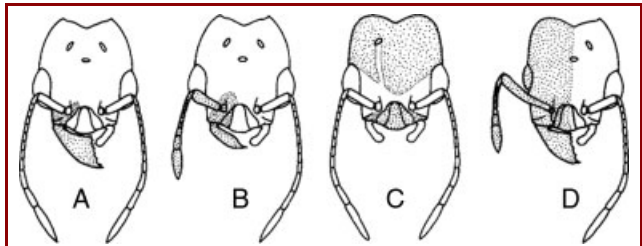


Figure 3. Gynandromorphs of *Vollenhovia emeryi* showing different forms of mosaicism in the head. Stippled areas show areas of female tissue in otherwise male-typical heads. The female mosaic patches are located in specific areas of the head that relate to certain adaptive traits, such as an ocelli, mandibles, and antennae, and may indicate location of various developmental mosaics within the head. Images taken from Kinomura and Yamauchi ('94).

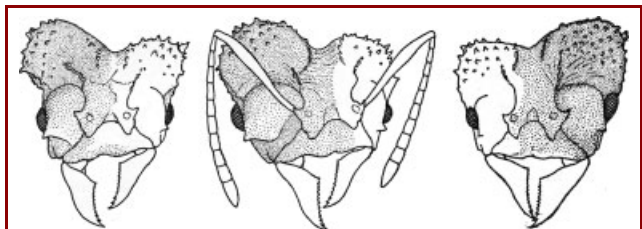


Figure 4. Three “intercaste” individuals showing the same form of head bilateral mosaicism as gynandromorphs. These intercastes of *Acromyrmex octospinosus* (Wheeler, '37) are between different morphs (worker vs. queen) within the female sex and, therefore, likely caused by environmental rather than “strictly genetic” factors.

In his foundational research into gynandromorphs, William M. Wheeler battled the impression that such variation was unimportant to biology because such mutants were "...monsters or 'freaks,' rare and exceptional..." ('37, p 36). Wheeler pointed out the need for closer surveys of whole colony samples for the presence of gynandromorphs and intercaste forms, which we believe will provide further insights on the potential evolvability of developmental modules. Gynandromorphs have been described, so far, from over 22 different genera of ants (Jones and Phillips, '85), and are more likely to be found if a concerted effort was made to discover them through whole colony surveys. Indeed, recent studies are finding these mutants are much more frequent in nature than has been typically assumed (Kinomura and Yamauchi, '94; Yoshizawa et al., 2009). In order to allow for more systematic analyses of gynandromorphic mutants, we have established an online database (<http://www.antweb.org/gynandromorph.jsp>) to collect and provide access to data and images of gynandromorphs and other mosaic form in ants. This database will hopefully draw attention to these mutants, and turn them from being marginal and underutilized observations to an additional and important source of data. Coupling such data with more extensive morphometric and developmental studies across a range of taxa will greatly enhance our understanding of the evolutionary developmental biology of both solitary and social insects.

ACKNOWLEDGMENTS

We thank Stefan Cover of the Museum of Comparative Zoology, Harvard University, for his help in confirming the identity of the specimens, and Ray Sanwald for his continuous support in collecting *Pheidole morrisoni* over the years. We thank Brian Fisher for helping us establish the AntWeb website database for gynandromorphic ants.

LITERATURE CITED

- Abouheif E, Wray GA. 2002. Evolution of the gene network underlying wing polyphenism in ants. *Science* 297:249–252.
- Akam M. 1998. Hox genes, homeosis and the evolution of segment identity: no need for hopeless monsters. *Int J Dev Biol* 42:445–451.
- Alonso C, Wilkins A. 2005. The molecular elements that underlie developmental evolution. *Nat Rev Genet* 6:709–715.
- Baker WK. 1978. A fine-structure gynandromorph fate map of the *Drosophila* head. *Genetics* 88:743–775.
- Berndt K-P, Kremer G. 1982. Heat shock-induced gynandromorphism in the pharaoh's ant, *Monomorium pharaonis* (L.). *Experientia* 38:798–799.
- Berndt K-P, Kremer G. 1983. New categories in the gynandromorphism of ants. *Ins Soc* 30:461–465.
- Carroll SB. 2008. Evo-devo and an expanding evolutionary synthesis: a genetic theory of morphological evolution. *Cell* 134:25–36.
- Carroll SB, Grenier JK, Weatherbee SD. 2005. From DNA to diversity: molecular genetics and the evolution of animal design. Malden, MA: Blackwell Pub.
- Cokendolpher JC, Francke OF. 1983. Gynandromorphic desert fire ant, *Solenopsis aurea* Wheeler (Hymenoptera: Formicidae). *J New York Entomol Soc* 91:242–245.
- Crawley WC. 1920. A gynandromorph of *Monomorium floricola*, Jerd. *Entomol Rec J Var* 32:217–218.
- Dietrich M. 2003. Richard Goldschmidt: hopeful monsters and other "heresies". *Nat Rev Genet* 4:68–74.
- Donisthorpe H. 1946. Fifty gynandromorphous ants taken in a single colony of *Myrmica sabuleti* Meinert. *Ireland Entomol (London)* 79:121–131
- Drescher W, Rothenbuhler W.C. 1963. Gynandromorph production by egg chilling: cytological mechanisms in honey bees. *J Hered* 54:195–201.
- García-Bellido A, Merriam JR. 1969. Cell lineage of the imaginal discs in *Drosophila* gynandromorphs. *J Exp Zool (Mol Dev Evol)* 170:61–75.
- Gilbert S. 2006. *Developmental biology*. 8th edition. Sinauer Associates, Inc.
- Gregg RE. 1938. Origin of castes in ants. *Ecology* 19:312–314.
- Haynie JL, Bryant PJ. 1986. Development of the eye-antenna imaginal disc and morphogenesis of the adult head in *Drosophila melanogaster*. *J Exp Zool (Mol Dev Evol)* 237:293–308.
- Heinze J, Trenkle S. 1997. Male polymorphism and gynandromorphs in the ant *Cardiocondyla emeryi*. *Naturwissenschaften* 84:129–131.
- Hoekstra H, Coyne J. 2007. The locus of evolution: evo devo and the genetics of adaptation. *Evolution* 61:995–1016.
- Hölldobler B, Wilson EO. 1990. *The ants*. Cambridge: Belknap Press.
- Hotta Y, Benzer S. 1970. Genetic dissection of the *Drosophila* nervous system by means of mosaics. *Proc Natl Acad Sci USA* 67:1156–1163.
- Irvine KD, Rauskolb C. 2001. Boundaries in development: formation and function. *Annu Rev Cell Dev Biol* 17:189–214.
- Jones SR, Phillips Jr SA. 1985. Gynandromorphism in the ant *Pheidole dentata* Mayr (Hymenoptera: Formicidae). *Proc Entomol Soc Washington* 87:583–586.
- Kamping A, Vaishali Katju V, Beukeboom LWH, Werren JH. 2007. Inheritance of Gynandromorphism in the parasitic wasp *Nasonia vitripennis*. *Genetics* 175:1321–1333.
- Khila A, Abouheif E. 2008. Reproductive constraint is a developmental mechanism that maintains social harmony in advanced ant societies. *Proc Natl Acad Sci USA* 105:17884–17889.
- Khila A, Abouheif E. 2010. Evaluating the role of reproductive constraints in ant social evolution. *Philos Trans R Soc B Biol Sci* 365:617–630.
- Kinomura K, Yamauchi K. 1994. Frequent occurrence of gynandromorphs in the natural population of the ant *Vollenhovia emeryi* (Hymenoptera: Formicidae). *Ins Soc* 41:273–278.
- Lawrence PA, Morata G. 1976. Compartments in the wing of *Drosophila*: a study of the engrailed gene. *Dev Biol* 50:321–337.
- Mavor JW. 1924. Gynandromorphs from X-rayed mothers. *Am Nat* 58:525–529.

- Michez D, Rasmont P, Terzo M, Vereecken NJ. 2009. A synthesis of gynandromorphy among wild bees (Hymenoptera: Apoidea), with an annotated description of several new cases. *Annales de la Societe Entomologique de France* 45:365–375.
- Morgan TH, Bridges CB. 1919. *Theory of gynandromorphs*. Washington: Carnegie Inst Publ 278:1–122.
- Morley DW. 1946. Ant gynandromorphs and other mosaics. *Nature* 157:741–742.
- Patterson JT. 1931. The production of gynandromorphs in *Drosophila melanogaster* by X-rays. *J Exp Zool (Mol Dev Evol)* 60:173–211.
- Pearson B, Child AR. 1980. The distribution of an esterase polymorphism in macrogynes and microgynes of *Myrmica rubra* Latreille. *Evolution* 34:105–109.
- Pie MR, Traniello JFA. 2007. Morphological evolution in a hyperdiverse clade: the ant genus *Pheidole*. *J Zool* 271:99–109.
- Provine WB. 1971. *The origins of theoretical population genetics*. Chicago: The University of Chicago Press.
- Ronshaugen M, McGinnis N, McGinnis W. 2002. Hox protein mutation and macroevolution of the insect body plan. *Nature* 415:914–917.
- Schlosser G, Thieffry D. 2000. Modularity in development and evolution. *BioEssays* 22:1043–1045.
- Shbailat SJ, Khila A, Abouheif E. 2010. Correlations between spatiotemporal changes in gene expression and apoptosis underlie wing polyphenism in the ant *Pheidole morrisi*. *Evol Dev* 12:579–590.
- Stern D. 2000. Perspective: evolutionary developmental biology and the problem of variation. *Evolution* 54:1079–1091.
- Taber SW, Francke OF. 1986. A bilateral gynandromorph of the Western harvester ant, *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae). *Southwest Nat* 31:274–276.
- Vandel A. 1931. Etude d'une gyandromorphe (dinergatandromorphe) de *Pheidole pallidula* Nyl. *Bull Bio France Belg* 65:114–129.
- von Dassow G, Meir E, Munro EM, Odell GM. 2000. The segment polarity network is a robust developmental module. *Nature* 406:188–192.
- Wagner GP, Altenberg L. 1996. Complex adaptations and the evolution of evolvability. *Evolution* 50:967–976.
- Wheeler WM. 1903. Some new gynandromorphous ants, with a review of the previously recorded cases. *Bull Am Mus Nat Hist* 19:653–683.
- Wheeler WM. 1919. Two gynandromorphous ants. *Psyche* 26:2–9.
- Wheeler WM. 1931. Concerning some ant gynandromorphs. *Psyche* 38:80–85.
- Wheeler WM. 1937. *Mosaics and other anomalies among ants*. Cambridge: Harvard University Press.
- Whiting PW. 1938. Anomalies and cast determination in ants. *J Hered* 29:189–193.
- Wilson EO. 1984. Tropical ant social parasites in the ant genus *Pheidole*, with an analysis of the anatomical parasitic syndrome (Hymenoptera: Formicidae). *Ins Soc* 31:316–334.
- Wray G. 2007. The evolutionary significance of cis-regulatory mutations. *Nat Rev Genet* 8:206–216.
- Yoshizawa J, Mimori K, Yamauchi K, Tsuchida K. 2009. Sex mosaics in a male dimorphic ant *Cardiocondyla kagutsuchi*. *Naturwissenschaften* 96:49–55.