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Perspectives

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The Emergence of Hymenopteran Genetics

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HYMENOPTERAN geneticists converged on the 42nd Annual Drosophila Research Conference held in Washington, DC, March 21–25, 2001, to participate in a workshop on the genetics of non-drosophilid insects and for a special satellite symposium on Hymenoptera. These were a result of a growing interest in the genetics of Hymenoptera, recognition of the need to integrate with Drosophila geneticists, and the desire to share information and methodology. It was clear from both meetings that the honeybee (*Apis mellifera*) and the parasitic wasp (*Nasonia vitripennis*) and its closely related congeners are emerging as important experimental organisms for genetics. In this article we show that there is a rich history in hymenopteran genetics spanning more than 150 years, with sex determination a common thread that has sometimes been intertwined with Drosophila. We also provide an overview of recent advances in hymenopteran genetics that set the stage for their emergence as important, tractable systems for understanding the genetics of sex determination, behavior, genome evolution, and the genetic basis of adaptive trait evolution.

THE ORIGINS OF HYMENOPTERAN GENETICS

Hymenopteran genetics began with Johann Dzierzon, a parish priest from the Prussian province of Silesia, now a part of Poland. In 1845, he published his hypothesis that male honeybees (drones) are derived from unfertilized eggs, whereas female-destined eggs are fertilized (Dzierzon 1845). This hypothesis met strong opposition from his beekeeping colleagues. However, his hypothesis was confirmed by Carl Th. von Siebold (1856), whose microscopic studies of eggs showed that drone-destined eggs contained no sperm. Nachtsheim's cytological studies (Nachtsheim 1913) settled the issue by demonstrating that eggs destined to be drones contained 16 chromosomes, whereas those of workers contained 32.

Gregor Mendel wanted to be a honeybee geneticist, but failed because he could not control the matings of queens and drones (Iltis 1924; Orel 1996). Mendel wanted to breed bees belonging to different races and take advantage of the effects of hybrid vigor for producing a better honey producer, a common theme throughout the history of bee breeding. Apparently he also wanted to use Dzierzon's discovery of haplodiploidy and look for segregation of color variants in the drones of hybrid queens derived from crosses of different races. This would have confirmed his theories of inheritance with an animal model. However, honeybee queens and males mate only while in free flight away from the nest. All of his attempts to manipulate their mating behavior failed. It is fortunate for biology that Mendel first bred garden peas.

Honeybee genetics began in earnest only with the development of artificial insemination (A.I.) technology. The history of the development of this technology spans more than 150 years (see Laidlaw 1987 for review). Near the end of the 18th century, the blind Swiss naturalist François Huber and his assistant François Burnens attempted to place the sperm of a male into the genital chamber of a queen by using a hair pencil, the first recorded attempt at artificial insemination of honeybees. These attempts failed. Many new and unsuccessful approaches at controlled mating were attempted in the ensuing 150 years until the efforts of Lloyd Watson, W. J. Nolan, Harry H. Laidlaw, Jr., and Otto Mackensen. Their efforts, spanning 30 years, resulted in the development of successful A.I. technology, control of honeybee mating, and honeybee breeding.

Breeding better bees was the primary objective of developing A.I. technology. In particular, honeybee breeders wanted to take advantage of the inbred-hybrid breeding methods that had proven so successful with hybrid corn production (Page and Laidlaw 1992; Crow

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1998). However, it became immediately apparent with inbreeding that something was seriously wrong with the honeybee genome, which resulted in high mortality of larvae and severely diminished the viabililty and productivity of inbred colonies. The answer came from the studies of P. W. Whiting on sex determination in the parasitic wasp, *Bracon hebetor*.

SEX DETERMINATION IN PARASITIC WASPS

In the first sentence of an article published in 1918, P. W. Whiting wrote, "The problem with sex determination is nowhere of greater interest than in the Hymenoptera" (P. Whiting 1918, p. 250). In 1922, C. B. Bridges published his theory of genic balance for Drosophila sex determination (Bridges 1922) and soon after proclaimed, "To me sex determination in the bee is the outstanding unsolved puzzle . . ." (Bridges 1925). The problem was obvious: if the sex of an animal was the result of a balance of male- and female-determining genes on the sex chromosomes and autosomes, how could haploidy give rise to males? Whiting decided to tackle the problem and chose to work on the small parasitic wasp *Bracon hebetor* (then called *Habrobracon juglandis* or *Habrobracon brevicornis*), rather than the honeybee. This was a wise decision at the time because it is much more easily cultured, has a much shorter generation time, and has fewer chromosomes (10 *vs.* 16), making the genetics more tractable. In addition, Bracon, unlike honeybees, were easily mated in captivity.

In 1933, Whiting first proposed the complementary sex determination (CSD) hypothesis on the basis of his experiments with *Bracon hebetor* (P. Whiting 1921, 1933). His theory was based on the regular occurrence of diploid males (then called biparental males) when *B. hebetor* was inbred. Whiting summarized his model in the following way: "There are different males similar in appearance but containing different sex factors and that the diploid complex of the female represents the heterozygous double dominant combination of two different male complexes" (P. Whiting 1943, p. 365). Subsequently, he demonstrated that multiple alleles exist at a single sex locus and segregate in populations. Females are always heterozygous, while individuals that are haploid or homozygous for the sex locus are males.

SEX DETERMINATION IN BEES

Early genetic studies of honeybees by using artificial insemination strongly suggested that they too, like Bracon, had a single locus system of sex determination. Otto Mackensen (1951), a research scientist for the U. S. Department of Agriculture, mated virgin queens to drones derived from their mother and found that half of the colonies had what was called "shot brood" resulting from the inviability of about half of the young larvae. He suspected it was the result of homozygosity at the sex locus, resulting in the nonhatching of eggs destined to be diploid males. Jerzy Woyke (1963), however, demonstrated that the death occurred during the first hours after hatching and that it was the result of the larvae being eaten by the adult worker honeybees, not lethal genes. Woyke developed a method to raise diploid male larvae and protect them from cannibalism (see Woyke 1986 for review). Larvae protected from cannibalism during the first 72 hr were able to complete development and became viable, but sterile, adults. Histological and cytological studies confirmed the existence of diploid males in honeybees.

Mackensen (1955), Laidlaw *et al.* (1956), and Adams *et al.* (1977) estimated that 11 to 17 different sex alleles were segregating in the populations they studied. Diploid males have subsequently been found in many species of Hymenoptera (Cook 1993) and have been taken as evidence for the widespread occurrence of this method of sex determination. However, it was not until a linkage map of the honeybee was constructed and the sex locus was mapped by Hunt and Page (1994) and Beye *et al.* (1994) that the single-locus CSD of Bracon was confirmed in another species. Recently, the sex-determination locus was also mapped for a European bumblebee, *Bombus terrestris* (Gadau *et al.* 2001).

MAPPING THE SEX LOCUS

Hunt and Page (1994, 1995) completed the first comprehensive hymenopteran genome mapping study when they mapped in honeybees the gene responsible for single-locus CSD. The map was based on the colony level phenotype, shot brood. Honeybees raise their larvae and pupae in individual cells on wax combs. The larvae are covered with a wax capping just prior to pupating. Colonies that produce diploid males, owing to shared sex alleles between the diploid queen and one or more of her haploid drone mates, have irregular patterns of capped brood resulting from the removal by the workers of the diploid male larvae. When a queen mates just one time and shares a sex allele in common with her haploid mate, half of the diploid offspring are male and are removed, leaving a distinct pattern of open brood cells. Hunt and Page produced drones from a queen (the drone mother) that were used to instrumentally inseminate sister queens of the drone mother. Approximately 25% of the sister test queens shared a sex allele in common with the drones, resulting in the production of shot brood. A genomic map was constructed by using the drones that, being haploid, represented individual meioses in the queen, and colony phenotypes were assigned to them. The map consisted of 365 random amplified polymorphic DNA (RAPD) markers distributed on 26 linkage groups spanning more than 3450 centimorgans (cM). Linkage analysis with the shot brood phenotype revealed a single genomic region responsible for this phenotype. This region was indepen-

dently confirmed with segregation of diploid males and workers by using a marker, the Q locus, that was closely linked at about 1.6 cM. This map not only revealed the map location of the CSD gene; it also demonstrated that the honeybee has an extremely large amount of recombination, about 52 kb/cM (or about 19 cM/Mb, 20 times the rate in humans), giving it the highest known rate of recombination of any higher eukaryote.

Beye *et al.* (1994) used multilocus fingerprinting and found a marker that was also linked to the gene for CSD. They designated their marker the Z locus. The two research groups joined forces, and it was subsequently found (Beye *et al.* 1999) that the Z and Q marker loci flanked the sex gene. Physical mapping of this region also confirmed the high recombination rate reported by Hunt *et al.* (1995), with an estimate of 44 kb/cM in this region (Beye *et al.* 1999).

IDENTIFYING THE SEX LOCUS

With flanking markers, Hasselmann *et al.* (2001) began to isolate the CSD gene by fine-scale mapping. Different cloning attempts demonstrated that the DNA of the sex-determining region was highly unstable in different cloning vectors, making it difficult to isolate. Therefore, a chromosome walk using a sex locus-enriched partial λ library was initiated (Beye *et al.* 1999). Progress of the chromosome walk was monitored with fine-scale mapping procedures (Hasselmann *et al.* 2001). A cloned region was finally isolated where no recombination was detected between markers and the sex locus in a sample of 1000 individuals. Because of the high recombination rate, the marker was estimated to be within about 5 kb of the sex locus. Cloned fragments were sequenced and found to contain several predicted exons. RT-PCR of early embryonic stages of bees suggested that some of these exons are coding for a single gene about 1.5 kb in length (M. Beye, M. Hasselmann, M. K. Fondrk and B. E. Page, unpublished results). Sequence comparisons suggest that the gene is a novel member of protein/RNA binding proteins, with weak homology to protein/RNA binding proteins in Drosophila that are involved in splicing regulation. These preliminary data suggest that sex determination in the Hymenoptera may be regulated at the level of differential splicing events as found for Drosophila.

A comparative analysis of genes involved in sex determination in a genic balance system like Drosophila and a haplodiploid system like Apis will provide new insight into the selective forces that both drive and constrain the evolution of sex-determining hierarchies. One of the most fascinating tasks remaining will be to determine how intracellular processes are affected by the large number of different sex alleles and their hetero- and homozygous combinations.

LINKAGE MAPS AND COMPARATIVE GENOME SIZES

The first detailed linkage map was based on *Drosophila melanogaster* and contained 111 mutant markers (Morgan *et al.* 1925). This map was difficult to construct because it was based on naturally occurring mutations that "popped up" in laboratory stocks. However, in 1927, Muller published his seminal paper on the use of radiation to induce mutations in Drosophila (Muller 1927), which meant a quantum leap for genetic studies and for constructing genetic linkage maps (Crow and Abrahamson 1997). The first linkage map for a hymenopteran species followed soon after. Muller suggested to P. W. Whiting even before the announcement of his results that similar studies should be done with *B. hebetor* (A. Whiting 1961, p. 316). This suggestion led to Whiting's studies of radiation-induced mutations in *B. hebetor*, which he reported in 1928 and 1929 (P. Whiting 1928, 1929). These radiation studies led in turn to a linkage map for *B. hebetor* that contained 35 genes and the sex locus (Anderson and Whiting 1939). In 1961, Anna Whiting (the wife of P. W. Whiting) summarized all linkage information available at that time and presented a map containing 38 mutant markers, and the sex locus, in eight linkage groups. The map spanned 585 cM, almost twice the size of an average *D. melanogaster* map (A. Whiting 1961).

In 1947, after studying *Bracon hebetor* for 30 years, Whiting decided to "devote the next 30 years" to Nasonia (A. Whiting 1961), another very distantly related small parasitic wasp. It became clear very quickly that Nasonia (a genus with three very closely related species) does not have single-locus CSD because diploid males were not produced even with severe inbreeding, thus showing that single-locus CSD is not universal within the Hymenoptera. It also became apparent that Nasonia was an excellent animal for laboratory studies because of its short generation time, few chromosomes ($n = 5$), the availability of numerous inbred and mutant strains, and the production of viable and fertile hybrids. Soon after switching to Nasonia, Whiting's group published a linkage map for a second hymenopteran species, *N. vitripennis*, which contained 14 mutant markers (Saul and Kayhart 1956). In 1993 Saul summarized all linkage information and published a *N. vitripennis* map containing 47 mutant markers (Saul 1993). This map comprised five linkage groups and spanned 264 cM, close to the size of *D. melanogaster*.

Hunt and Page (1995) published the first detailed linkage map for an insect other than Drosophila with RAPD markers. The use of DNA markers greatly accelerated the construction of maps and resulted in higher levels of marker saturation than were possible with visible mutant markers. The new relative ease of constructing linkage maps resulted in several new maps and the ability to conduct comparative studies of recombination. The map of the honeybee was followed by those of the

bumblebee, *Bombus terrestris* (Gadau *et al.* 2001), and of five species of parasitic hymenoptera: *Bracon hebetor* (Antolin *et al.* 1996), *Bracon* near *hebetor* (Holloway *et al.* 2000), *N. vitripennis* × *N. giraulti* (Gadau *et al.* 1999), and *Trichogramma brassicae* (Laurent *et al.* 1998). The linkage map of the honeybee was huge—more than 3450 cM—when compared with all of these (Gadau *et al.* 2000). Gadau *et al.* (2001) also introduced a new method for constructing maps from haploid male progeny derived from females without knowing the parental linkage phase of the markers. This technique provides the opportunity to construct maps for species where controlled mating is not possible.

Why do honeybees have such a large recombinational map? Hunt and Page (1995) hypothesized that this high recombination frequency might be related to male haploidy and/or small chromosome size. However, relative genome sizes of male haploid parasitic Hymenoptera range from 829 to 1330 cM, making that an unlikely explanation (Gadau *et al.* 2000). *Bombus terrestris*, like honeybees, has many small chromosomes ($n = 18$). However, *B. terrestris* has a relative small recombinational genome of 1091 cM. An alternative hypothesis is that high levels of recombination result from social life histories. Again, a comparison with the highly social bumblebee invalidates this argument. So, for now the high recombination rate of the honeybee remains an enigma.

QUANTITATIVE GENETICS OF HYMENOPTERA

The ability to rapidly construct linkage maps has also offered opportunities to map quantitative trait loci (QTL) associated with naturally occurring traits of Hymenoptera. To date, QTL have been found in honeybees for several traits relating to foraging behavior (Hunt *et al.* 1995; Page *et al.* 2001), colony defense (Hunt *et al.* 1998), and learning (Chandra *et al.* 2001), demonstrating the role of major genes in naturally occurring behavioral variation. Morphological traits have been mapped for honeybees (Hunt and Page 1995; Hunt *et al.* 1998, 1999), *Bombus terrestris* (J. Gadau, unpublished results), and an interspecific hybrid of *Nasonia vitripennis* and *N. giraulti* (J. Gadau, R. E. Page and J. H. Werren, unpublished results). Maps of the Nasonia interspecific cross have also revealed the role of epistasis in shaping adaptive differences in morphology (Gadau *et al.*, unpublished results) and the role of epistasis in hybrid breakdown (Gadau *et al.* 1999).

HYMENOPTERA AS GENETIC MODELS

Drosophila genetics has provided many tools and concepts that are applicable to hymenopteran genetic research. This was true for P. W. Whiting and continues today. Many of the new genomic tools available for Drosophila are now becoming available for honeybees. For example, the honeybee community shares cDNA, plasmid, phage, cosmid, and BAC libraries. DNA sequence data from Drosophila are being used to isolate homologous honeybee genes from these libraries. This has proven especially useful in honeybee neurobiology for studying signal transduction pathways (see Blenau *et al.* 2000) and diurnal rhythms correlated with *period* (Toma *et al.* 2000). Expressed sequence tags (ESTs) are being developed in several labs (Whitfield *et al.* 2002; see http://keck1.biotec.uiuc.edu/bee/honeybee_project.htm) and have been used in studies of honeybee caste determination and development (Evans and Wheeler 2000).

The biology of the honeybee remains the biggest impediment to the application of many genetic techniques developed with model organisms; it is not a laboratory animal. Generation times are long, culturing of reproductives is labor intensive, breeding populations are small, and the effects of inbreeding are severe, precluding the development of isogenic lines of bees. However, there is no doubt that the rich behavioral repertoire of honeybees will continue to excite biologists and motivate them to apply genetic techniques to better understand their social behavior.

Nasonia is most like Drosophila with respect to its genetics and culturing methods and, therefore, the most likely hymenopteran genetic-model organism. It has just five chromosomes and the generation times are short, about 15 days. A single female can produce more than 200 reproductive offspring. They can be cultured in the laboratory in small containers. Isogenic lines are available for three species: *N. vitripennis*, *N. giraulti*, and *N. longicornis*. Lines exist where the nuclear genome of one species has been introgressed into the cytoplasm of another, and recombinant inbred lines have been developed from hybrid crosses of *N. vitripennis*, *N. longicornis*, and *N. giraulti*. These are powerful tools for studying development, behavior, and morphology among these species.

Bracon was the model for single-locus CSD. However, the honeybee is leading the way in the quest for "the sex gene." Soon the honeybee sex-determining gene will be completely characterized. When finished, this will be a rare example of going from a naturally occurring phenotypic trait (in this case the colony phenotype of shot brood) to the gene by fine-scale mapping. Bracon, however, should still be very useful for comparative studies of complementary sex determination and other sex-determining factors that work together with CSD (Holloway *et al.* 2000).

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