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► **To cite this version:**

Amro Zayed. Bee genetics and conservation. *Apidologie*, 2009, 40 (2), 10.1051/apido/2009026 .
hal-00892012

HAL Id: hal-00892012

<https://hal.science/hal-00892012>

Submitted on 11 May 2020

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Bee genetics and conservation*

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Received 29 August 2008 – Revised 5 January 2009 – Accepted 15 January 2009

Abstract – The emerging threat of pollinator decline has motivated research on bee conservation biology in order to both understand the causes of declines and to develop appropriate conservation strategies. The application of genetics to the conservation of diploid animals has proven to be important for both overcoming genetic threats to population viability and for providing tools to guide conservation programs. However, the haplodiploid bees have several unusual genetic properties of relevance to their conservation, which warrant special attention. Here I review how haplodiploidy and complementary sex determination affect genetic parameters pertinent to the viability and future evolutionary potential of bee populations. I also review how genetic tools can improve the conservation management of bees. I find that bees are especially prone to extinction for genetic reasons, and that genetics can provide invaluable tools for managing bee populations to circumvent pollinator decline.

haplodiploid / complementary sex determination / inbreeding depression / diploid males / extinction

1. INTRODUCTION

There are nearly 20,000 known species of bees worldwide (Michener, 2000), and they are integral components of terrestrial ecosystems due to their indispensable role as pollinators (Allen-Wardell et al., 1998; Klein et al., 2007; Kremen et al., 2007). Over the past decade, multiple lines of evidence have shown that both native and managed pollinators are experiencing a seemingly world-wide decline (Biesmeijer et al., 2006; Fitzpatrick et al., 2006; Kosior et al., 2007; NRC, 2007; Colla and Packer, 2008; Goulson et al., 2008; Grixti et al., 2009), fueling both ecological and economic concerns (Kremen et al., 2002; Fontaine et al., 2006; Vamosi et al., 2006; Olesen et al., 2007; Pauw, 2007). This emerging threat has mobilized the academic community through the establishment of international research initiatives and partnerships with the

purpose of examining the causes of pollinator decline and developing appropriate conservation strategies to circumvent it (e.g. Dias et al., 1999; Kremen et al., 2007; NRC, 2007; Byrne and Fitzpatrick, 2009).

Several extrinsic factors have been proposed to explain the observed declines in bee populations: habitat fragmentation and loss, agricultural intensification, overuse of pesticides, pathogen spill-over from managed pollinators, invasive species, and global climate change (Cane, 2001; Cane and Tepedino, 2001; Kremen et al., 2002; Müller et al., 2006; NRC, 2007; Murray et al., 2009). Genetic aspects of bee declines were completely neglected in the early syntheses (e.g. Dias et al., 1999) despite the overwhelming evidence that genetic factors can play important roles in species extinction (Saccheri et al., 1998; Frankham et al., 2002; Spielman et al., 2004; Hanski and Saccheri, 2006). Several factors likely contributed to the initial oversight of possible genetic aspects of bee declines, including: (1) Prior theoretical

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arguments suggesting that genetics is relatively unimportant in the conservation biology of haplodiploids (Box I). (2) Difficulties in extrapolating knowledge gained from diploid organisms to the haplodiploid bees. (3) Lack of studies examining how specific bee genetic and life history traits (e.g. haplodiploidy, complementary sex determination, sociality, etc.) impact population viability. (4) Lack of genetic resources for bees. However, theoretical and technical advances over the past decade have overturned longstanding views regarding the immunity of bees to genetic threats in small populations and prompted research on their conservation genetics.

Research in conservation genetics can be conceptually divided into two general types of inquiry: (1) How does genetics contribute to population decline and extinction? (2) How can molecular tools be utilized to learn about an organism's characteristics relevant to conservation and management (e.g. taxonomy, natural history, and demography)? In this review, I summarize and synthesize studies on how genetics can contribute to bee declines (Sect. 2), focusing on how haplodiploidy and genetic sex determination affect parameters relevant to population viability and evolutionary potential. I also review how genetic and genomic tools can be used in conservation and management of bee populations (Sect. 3). To ensure that my review is accessible to the largest possible readership, I focus on presenting general concepts without delving into detailed mathematical treatments. For the mathematically inclined, I recommend several reviews on haplodiploid population genetics (Crozier, 1977; Hedrick and Parker, 1997), and conservation genetics (Frankham et al., 2002; Gaggiotti, 2003; Hedrick, 2004; Frankham, 2005).

2. GENETIC ASPECTS OF BEE DECLINES

Genetic threats can reduce the viability of small wildlife populations over the short term by reducing fitness, as well as over the long term by limiting evolutionary potential (Box I) and adaptability. Here I discuss the three most

plausible and best supported genetic threats to the viability of small bee populations: Complementary sex determination, inbreeding depression, and loss of genetic diversity and consequent evolutionary potential. I do not discuss the hypothesized effects of the accumulation of deleterious mutations on population viability (Lynch et al., 1995), given limited empirical support of 'mutational meltdowns' in sexually-reproducing populations (reviewed by Frankham, 2005). Box I contains a glossary of terms relevant to bee conservation genetics. The terminology relating to inbreeding is historically a very murky subject (Jacquard, 1975; Templeton and Read, 1994; Keller and Waller, 2002). Following Glémin (2003) and Leberg and Firmin (2008), here I use 'systematic inbreeding' to describe species with mating systems characterized by 'inbreeding' (Box I). I use inbreeding by drift to describe the increasing probability of relatedness among mates in small populations (Box I). The term inbreeding depression (Box I) can also be ambiguous. Defined broadly, inbreeding depression, or reduced fitness of inbred individuals, can encompass the effects of complementary sex determination that are unique to some members of the Hymenoptera. However, inbreeding depression is classically ascribed to dominance and overdominance (Box I) – which are common to both diploids and haplodiploids. Here I treat the unique effects of complementary sex determination on the fitness of haplodiploids as distinct from inbreeding depression caused by dominance and overdominance.

2.1. Complementary sex determination and bee declines: death by diploidy

Bees belong to the insect order Hymenoptera, a group characterized by haplodiploidy (i.e. Females are diploid while males are usually haploid; Box I). The presumed ancestral sex determination mechanism in the Hymenoptera is single locus complementary sex determination (sl-CSD; Box I), where sex is determined by genotype at a single gene (Cook, 1993; van Wilgenburg et al., 2006; Heimpel and de Boer, 2008).

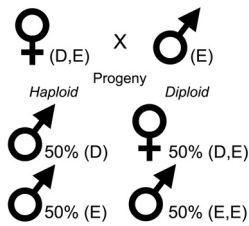


Figure 1. Diploid male production. A matched mating results when a female mates with a male sharing a sex-determining allele in common (allele E). Half of the female's diploid progeny – all intended to be female – will be homozygous at the sex determination locus and will develop into diploid males (genotype EE).

Heterozygotes at the sex-determination locus develop into diploid females from fertilized eggs, while hemizygotes develop into haploid males from unfertilized eggs. In cases where a female mates with a haploid male that shares a sex-determining allele in common (i.e. matched mating; Box I) half of the female's diploid progeny will be homozygous at the sex-determination locus and will consequently develop into diploid males instead of females (Fig. 1). Diploid males have been observed in 4 families and at least 27 species of both solitary and social bees (Tab. I), and the sex determination locus has been molecularly identified in the honey bee *Apis mellifera* (Beye et al., 2003) and genetically mapped in the bumble bee *Bombus terrestris* (Gadau et al., 2001).

The production of diploid males represents a large genetic cost in bee populations. Diploid males are mostly either inviable or sterile (Agoze et al., 1994; Duchateau et al., 1994; Holloway et al., 1999; Liebert et al., 2004, 2005; Heimpel and de Boer, 2008). Female Hymenoptera only fertilize their eggs when attempting to produce daughters. The production of diploid males from fertilized eggs therefore acts to increase female mortality. A secondary cost to diploid male production is also incurred if diploid males are viable and achieve matings. Viable diploid males produce diploid sperm and thus females mating with them produce inviable fertilized eggs or sterile triploid daughters (Krieger et al., 1999;

Ayabe et al., 2004; Liebert et al., 2004, 2005), with one rare exception in an aculeate wasp (Cowan and Stahlhut, 2004). In such cases, the production of diploid males indirectly increases female mortality by constraining their mates to the production of haploid males and triploid daughters. Therefore, diploid male production increases female mortality over one or two generations, given inviable or effectively sterile diploid males respectively. Ultimately, increased female mortality caused by diploid male production will result in reduced population growth rates in bee populations (Stouthamer et al., 1992; Pamilo and Crozier, 1997). In social bees, diploid male production will effectively increase female mortality for both reproductive and worker castes, and can thus reduce both population and colony growth rates (Cook and Crozier, 1995). In cases where colony survival is a function of the size of the worker force, diploid male production can significantly increase colony mortality (Plowright and Pallett, 1979; Ross and Fletcher, 1986). Diploid male production is thus expected to be costly in both solitary and social bees.

The number of sex-determining alleles, which indirectly controls the frequency of diploid male production, is an increasing function of population size (Fig. 2). Mutation provides a constant but slow (e.g. 10^{-6} , Kerr, 1997) input of novel sex-determining alleles into the gene pool, increasing genetic diversity at the sex-determination locus. Given the fitness costs associated with the production of homozygotes at the sex-determination locus, which develop into diploid males, the locus experiences strong negative frequency dependent selection (Box I): New sex-determining alleles introduced by mutation will initially rise in frequency since individuals carrying them have higher relative fitness (i.e. they are less likely to participate in matched-matings). However, as selection increases the frequency of the new allele, relative to pre-existing alleles, the relative fitness of individuals carrying the new allele will diminish as they participate in more matched-matings. Negative frequency dependent selection therefore acts to homogenize the frequencies of sex-determining alleles in a population: The equilibrium allele frequency at the sex-determining

Table I. Bee species where diploid males have been reported.

Species	Reference
Family Andrenidae	
<i>Andrena scotica</i>	(Paxton et al., 2000)
Family Halictidae	
<i>Augochlorella striata</i>	(Packer and Owen, 1990)
<i>Halictus poeyi</i>	(Zayed and Packer, 2001)
<i>Lasioglossum leucozonium</i>	(Zayed et al., 2007)
<i>Lasioglossum zephyrum</i>	(Kukuk and May, 1990)
Family Megachilidae	
<i>Megachile rotundata</i>	(McCorquodale and Owen, 1994)
Family Apidae	
<i>Apis cerana</i>	(Woyke, 1979)
<i>Apis mellifera</i>	(Adams et al., 1977; and others)
<i>Bombus atratus</i>	(Plowright and Pallett, 1979)
<i>Bombus florilegus</i>	(Takahashi et al., 2008)
<i>Bombus impatiens</i>	Zayed et al. unpublished
<i>Bombus muscorum</i>	(Darvill et al., 2006)
<i>Bombus sylvarum</i>	(Ellis et al., 2006)
<i>Bombus terrestris</i>	(Duchateau et al., 1994)
<i>Eufriesea magrettii</i>	(Lopez-Uribe et al., 2007)
<i>Euglossa imperialis</i>	(Zayed et al., 2004)
<i>Euglossa mandibularis</i>	(Takahashi et al., 2001)
<i>Euglossa meriana</i>	(Roubik et al., 1996)
<i>Euglossa piliventris</i>	(Lopez-Uribe et al., 2007)
<i>Euglossa sapphirina</i>	(Roubik et al., 1996)
<i>Euglossa tridentata</i>	(Roubik et al., 1996)
<i>Eulaema cingulata</i>	(Lopez-Uribe et al., 2007)
<i>Melipona compressipes</i>	(Kerr, 1987)
<i>Melipona scutellaris</i>	(Carvalho, 2001)
<i>Melipona quadrifasciata</i>	(Camargo, 1979)
<i>Scaptotrigona postica</i>	(Paxton et al., 2003)
<i>Trigona carbonaria</i>	(Green and Oldroyd, 2002)

locus is $1/k$, where k represents the number of sex-determining alleles in the population (Adams et al., 1977; Yokoyama and Nei, 1979; Owen and Packer, 1994). In a random mating population, the frequency of diploids that are male, measured as the proportion of homozygotes at the sex-determination locus, is $k(1/k)^2$ (i.e. number of sex-determining alleles multiplied by the expected homozygosity of an allele at equilibrium frequency) =

$1/k$. The frequency of diploid male production is thus equivalent to the equilibrium frequency of sex-determining alleles, and thus diploid male production is inversely proportional to the number of sex-determining alleles in the population (Fig. 2). Ultimately, the number of sex-determining alleles in a population is a function of mutation, negative frequency dependent selection, and genetic drift (Yokoyama and Nei, 1979; Cornuet,

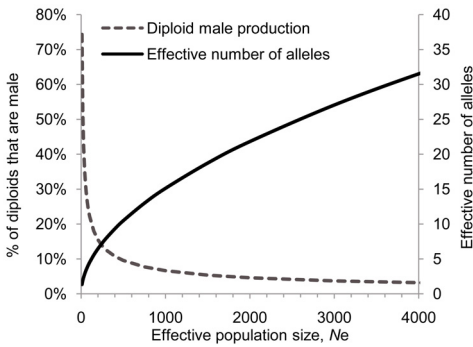


Figure 2. Smaller populations maintain less alleles at the sex determination locus, and consequently produce higher frequencies of diploid males, when compared to larger populations. The graph was obtained assuming a mutation rate of 10^{-6} using Cornuet's (1980) mutation-selection-drift equilibrium model.

1980). Smaller populations are more affected by drift and are thus expected to maintain fewer sex-determining alleles, and have higher frequencies of diploid male production compared to larger populations (Fig. 2).

Complementary sex determination and diploid male production are expected to have a negative impact on the population viability of bees. The production of diploid males is expected to be higher in smaller populations (Fig. 2), and is thus expected to reduce growth rates in these populations (Stouthamer et al., 1992; Pamilo and Crozier, 1997). Zayed and Packer (2005) explored the relationship between diploid male production and extinction using simulation models of population viability. Population viability analysis is a very useful framework to examine how deterministic and stochastic events impact extinction risk (Brook et al., 2002a). Briefly, Zayed and Packer (2005) modelled hypothetical haplodiploid populations assuming a wide range of population sizes, and growth rates. First, simulations were conducted assuming no complementary sex determination to estimate the risk of extinction due to demographic factors alone. Then, simulations incorporating complementary sex determination were performed to examine the effects of diploid male production on extinction risk. Given initially identi-

cal population sizes and growth rates, the production of diploid males increased the risk of extinction by more than an order of magnitude on average (Zayed and Packer, 2005). The production of effectively sterile diploid males increased the extinction risk over that caused by inviable diploid males, because of the former's secondary effects on population growth rates. In diploid species, inbreeding depression is the biggest genetic threat to short-term population viability (Brook et al., 2002b; Spielman et al., 2004). Zayed and Packer (2005) also compared the probability of extinction caused by inbreeding depression in diploid populations with that caused by diploid male production in haplodiploid populations. Given identical starting population parameters, the risk of extinction caused by diploid male production in haplodiploid populations was an order of magnitude higher than that caused by inbreeding depression in diploid populations, making complementary sex determination in haplodiploids the largest known genetic threat to population viability (Zayed and Packer, 2005; Hedrick et al., 2006).

Zayed and Packer (2005) attributed this increased extinction risk to the effects of the "Diploid Male Vortex". The production of diploid males can initiate a positive feedback cycle that leads to rapid extinction. This occurs because the production of diploid males initially reduces population sizes and growth rates. Demographic and environmental stochasticity combined with increased genetic drift in a smaller population further reduce the number of sex-determining alleles which leads to higher levels of diploid male production. This diploid male vortex will continue to reduce the proportion of females in the population, further reducing growth rates. In isolated populations with small sizes and/or low reproductive rates, negative population growth rates can be rapidly achieved, ultimately leading to extinction.

Although it is difficult to attribute specific causes to population declines and extinction in nature, several lines of evidence suggest that diploid male production can contribute, and has contributed, to bee declines:

1. Higher levels of extinction are observed in laboratory populations of parasitoid wasps

with versus without complementary sex determination (Stouthamer et al., 1992; Wu et al., 2003), as expected from Zayed and Packer's (2005) simulations. Furthermore, when laboratory populations of parasitoid wasps go extinct, they usually do so with high male-biased sex ratios (Simmonds, 1947), as predicted by Zayed and Packer's (2005) simulations.

2. The simulation models developed by Zayed and Packer (2005) show that diploid male production can bring-about extinction over realistic demographic parameters for bees. In simulations, the net reproductive output of female bees was an important predictor for their susceptibility to extinction through the diploid male vortex. Many solitary bee species have very low life-time fecundity (Michener and Rettenmeyer, 1956; Danks, 1971; Else et al., 1978; Minckley et al., 1994; Franzén and Larsson, 2007). Franzén and Larsson (2007) recently summarized the reproductive output of several *Andrena* species which averaged 5.8 eggs per lifetime. At these levels, the risk of extinction caused by the diploid male vortex are extremely high for a small population unless the environment is conducive to rapid population growth (Zayed and Packer, 2005).

3. The production of diploid males has been empirically shown to increase colony mortality for some social Hymenoptera, both in the laboratory (Plowright and Pallett, 1979) and in the field (Ross and Fletcher, 1986).

4. Diploid males have been found in many declining and/or endangered solitary and social bee species (Carvalho, 2001; Zayed et al., 2004; Darvill et al., 2006; Ellis et al., 2006; Takahashi et al., 2008). For example, diploid males, and triploid queens have been observed in small populations of the rare and locally distributed bumble bee *Bombus florilegus* (Takahashi et al., 2008). In the stingless bee *Melipona scutellaris*, small isolated populations maintain low numbers of sex-determining alleles and rapidly go extinct (Carvalho, 2001), presumably due to the effects of the diploid male vortex. However, these populations can be 'rescued' through the intentional introduction of mated queens from other populations, which increases the number of sex-determining alleles (Carvalho, 2001).

Carvalho's (2001) study demonstrates a direct causal link between diploid male production, and extinction in bees.

The above lines of evidence suggest that diploid male production can be, and likely has been, involved in the global decline of bees. Habitat loss and fragmentation are expected to reduce levels of genetic variation in natural populations (Gilpin, 1991; Hedrick and Gilpin, 1997; Whitlock and Barton, 1997), including allelic variation at the sex-determination locus, making smaller bee populations more susceptible to extinction through the diploid male vortex. Furthermore, any extrinsic factor that acts to reduce bee population growth rates (e.g. overuse of pesticides, pathogen/parasite infections, competition from invasive species) will also, through the dependence of diploid male production on population size, initiate the diploid male vortex (Fig. 3). Bee populations are thus expected to decline faster, and recover slower, than expected based on ecological predictions alone due to the synergistic negative effects of diploid male production on population size and growth (Fig. 3).

Stochastic models supporting the role of diploid male production in bee declines have assumed monandry (i.e. females singly mate), random mating, and no adaptations to reduce the costs of diploid male production (Zayed and Packer, 2005). The first two assumptions are well supported by empirical data (Eickwort and Ginsberg, 1980; Estoup et al., 1995; Strassmann, 2001; Green and Oldroyd, 2002; Palmer et al., 2002; Paxton et al., 2003; Cameron et al., 2004; Paxton, 2005; Beveridge et al., 2006; Kraus et al., 2008), with some exceptions (e.g. Page, 1980; Paxton et al., 2000). Polyandry is expected to reduce between-family variation in the production of diploid males when compared to monandry. However, the total frequency of diploid male production will remain unchanged (Cook and Crozier, 1995). Lower variance in diploid male frequencies between families should slightly reduce the effects of drift (Frankham, 1995a), although similar extinction risks should be experienced under both polyandry and monandry given equal population-wide frequencies of diploid male

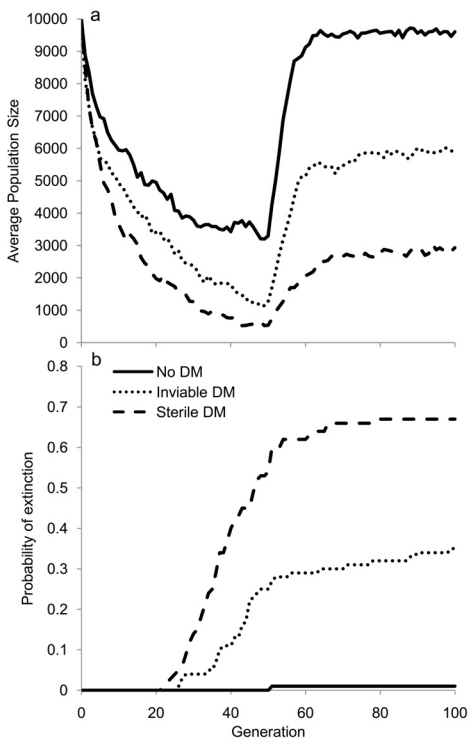


Figure 3. Complementary sex determination synergistically interacts with extrinsic factors resulting in faster decline and slower recovery of bee populations. I stochastically modelled (Zayed and Packer, 2005) a bee population ($N = 10\,000$ bees) experiencing negative growth rates (assumed $r = -0.28$) caused by an extrinsic environmental factor. At generation 50, the extrinsic factor was removed allowing the population to recover (assumed $r = 0.28$). The population size (a), averaged over 100 simulation iterations, and the probability of extinction (b), are presented for populations without diploid male (DM) production, as well as with DM production assuming inviable or effectively sterile DMs. The production of diploid males reduced growth rates below that caused by the extrinsic factor resulting in faster decline and slower recovery (a).

production. Polyandry in social bees may serve to reduce the cost of diploid male production on colony mortality, but this largely depends on the shape of the relationship between colony fitness and diploid male production (Cook and Crozier, 1995). Systematic Inbreeding (Box I) – a rare phenomenon in bees which have several adaptations that promote

random mating (Eickwort and Ginsberg, 1980) – is expected to increase diploid male production and the risk of extinction over the predictions of Zayed and Packer's (2005) models that assume random mating. For example, 50% of matings between siblings given monandry will be matched even in a population with a large number of sex-determining alleles. Hymenopteran species with mating systems characterized by systematic inbreeding tend not to have sl-CSD (van Wilgenburg et al., 2006; Heimpel and de Boer, 2008), and this may be also expected of bees with similar mating systems. However, sl-CSD has never been plausibly rejected in any bee species examined so far (van Wilgenburg et al., 2006), although the available data are somewhat limited in taxonomic scope with a predominance of studies on the Family Apidae (Tab. I). Frequencies of diploid males estimated from samples of adults in the systematically inbreeding *Andrena scotia* were lower than expected given sl-CSD, suggesting that this species may have multiple-locus CSD or a related adaptation to reduce levels of diploid male production (Paxton et al., 2000). However, a lower frequency of diploid males, sampled as adults, does not necessarily rule out sl-CSD given the potential for high pre-adult mortality of diploid males (Cook and Crozier, 1995; van Wilgenburg et al., 2006; Heimpel and de Boer, 2008).

Finally, it has been proposed that hymenopterans with sl-CSD should evolve adaptations to reduce the cost of diploid male production (Cowan and Stahlhut, 2004; Paxton, 2005; van Wilgenburg et al., 2006). Such adaptations include the evolution of more sex loci, restored fertility of diploid males, selective fertilization of non-matched sperm in polyandrous females, avoidance of matched matings through sex-allele signaling, and others (reviewed by Cook and Crozier, 1995; van Wilgenburg et al., 2006). Evidence for most of these hypotheses is either rare (Cowan and Stahlhut, 2004 report reproductive diploid males in an aculeate wasp; De Boer et al., 2007 report multiple locus CSD in a parasitoid wasp), or completely lacking (Cook and Crozier, 1995; van Wilgenburg et al., 2006). In the largely random-mating bees, it is unclear if

adaptations reducing the cost of diploid male production would arise in normally large populations where diploid males are rare and the costs of diploid male production are low. Empirical work is needed to investigate the possibility that bees have adaptations which reduce the cost of diploid male production, and theoretical work is needed to derive conditions under which such adaptations can arise. The limited evidence suggests that such adaptations do not exist, or are not widespread.

In summary, there is compelling theoretical and empirical evidence that diploid male production can substantially reduce the viability of small bee populations. The diploid male vortex is also expected to synergistically interact with other deterministic factors causing faster extinction rates than would be expected. Endangered bee species targeted for conservation should be managed to reduce frequencies of diploid male production. This can be achieved by promoting gene flow between populations (Zayed, unpubl. data), and attempting to maintain a high level of allelic diversity at the sex determination locus in bee meta-populations.

2.2. Haplodiploidy, inbreeding depression, and bee declines

Inbreeding depression (i.e. reduction in fitness due to inbreeding) is considered the major threat to the short term viability of small populations of diploid organisms (Brook et al., 2002b; Gaggiotti, 2003; Spielman et al., 2004; Frankham, 2005). In small closed populations, inbreeding by drift is an unavoidable phenomenon as relatedness between individuals is expected to increase over time (Hedrick, 2000; Frankham et al., 2002). Inbreeding increases the frequency of homozygous genotypes and this is often associated with a reduction in fitness traits (Hedrick, 2000; Frankham et al., 2002). Inbreeding depression has been observed for a large number of mostly diploid organisms (Ralls and Ballou, 1983; Ralls et al., 1988; Keller and Waller, 2002; Armbruster and Reed, 2005) where it has been both theoretically and empirically shown to increase the risk of extinction in

small populations (Frankham, 1995b, 1998; Saccheri et al., 1998; Westemeier et al., 1998; Hedrick and Kalinowski, 2000; Brook et al., 2002b; Gaggiotti, 2003; Reed and Frankham, 2003; Spielman et al., 2004; Frankham, 2005; Vilas et al., 2006).

The exact cause of inbreeding depression is often debated among evolutionary biologists (Charlesworth and Charlesworth, 1999; Hedrick and Kalinowski, 2000; Crnokrak and Barrett, 2002; Keller and Waller, 2002; Leberg and Firmin, 2008). Two major hypotheses can mechanistically explain inbreeding depression. The dominance hypothesis (Box I) posits that inbreeding depression is caused by the expression of deleterious recessive alleles normally sheltered in heterozygous individuals. The overdominance (Box I) hypothesis states that inbreeding depression is caused by lower fitness of homozygous versus heterozygous genotypes. Inbreeding increases the frequency of homozygous genotypes, and reduced fitness of inbred individuals can be explained by both the dominance and overdominance hypotheses. Empirical data support the view that, generally, inbreeding depression results mostly from dominance with a minor contribution from overdominance (Charlesworth and Charlesworth, 1999; Hedrick and Kalinowski, 2000; Keller and Waller, 2002), although overdominance can be a major component of inbreeding depression for some traits (Charlesworth and Charlesworth, 1999).

In haplodiploids, inbreeding depression can result from dominance, overdominance, and from increased homozygosity at the sex determination locus (see Sect. 2.1). Here I ignore complementary sex determination as a source of inbreeding depression in haplodiploids since classically, inbreeding depression is defined as reductions in fitness of inbred individuals due to dominance and overdominance only. Several attempts have been made to theoretically predict the relative extent of inbreeding depression in haplodiploids (Crozier, 1976b, 1985; Werren, 1993; Hedrick and Parker, 1997). In haplodiploids, recessive lethal and mildly deleterious alleles are constantly exposed to selection and are more likely to be purged in haploid males, and the equilibrium frequency of these alleles is

therefore expected to be lower in haplodiploid versus diploid populations. If recessive deleterious mutations are the sole cause of inbreeding depression, then males, and females, in a haplodiploid population should exhibit higher, and lower, genetic load than either sex of a diploid population, respectively (Werren, 1993). Assuming the dominance hypothesis and an equal sex ratio, the genetic load of haplodiploids should be on average approximately 25% lower than diploid populations, although exact values depend on levels of selection against deleterious alleles, degree of dominance, and whether systematic inbreeding is practiced (Werren, 1993; Hedrick and Parker, 1997). Deleterious mutations in genes with effects limited only to females are not expected to be purged by selection on haploid males. The genetic load for sex-limited genes should thus be substantial in female haplodiploids (Crozier, 1976b), but will still be 25% lower than the sex-limited load of a diploid population (Werren, 1993). Finally, haplodiploidy is not expected to reduce the inbreeding depression caused by overdominance. The effects of haplodiploidy on inbreeding depression are clearly dependent on many important, but often unknown, parameters which include the genetic basis of inbreeding depression (dominance versus overdominance), the deleterious effects and relative dominance of mutations (e.g. lethal versus mild deleterious, recessive versus partially recessive), and the contribution of sex-limited mutations to the genetic load. Although it seems clear that haplodiploidy can reduce the genetic load under many circumstances, the magnitude of the expected reductions are small, implying that haplodiploid populations should still experience significant inbreeding depression.

Lowered fitness of inbred individuals has been documented in several social bees (e.g. Bienefeld et al., 1989; Gerloff and Schmid-Hempel, 2005). However, most studies of inbreeding depression in bees confound the negative effects of diploid male production with those caused by dominance and overdominance, and only the latter two contribute to inbreeding depression as classically defined. Parasitoid wasps without com-

plementary sex determination are appropriate organisms to investigate the consequences of haplodiploidy on inbreeding depression without the confounding effects of complementary sex determination. Henter (2003) and Antolin (1999) reviewed estimates of inbreeding depression in Hymenopteran parasitoids for several fitness traits. Both authors showed that haplodiploids without complementary sex determination experience substantial inbreeding depression, although lower than that experienced by diploids. Furthermore, even some haplodiploid species with systematically-inbreeding mating systems still experience inbreeding depression (Luna and Hawkins, 2004; Schremph et al., 2006). These results indicate that haplodiploidy does not offer complete immunity from inbreeding depression, and that both overdominance and dominance in female-limited genes likely constitute a significant source of inbreeding depression in haplodiploids. Inbred haplodiploid populations, including those of bees, are thus expected to suffer from reduction in fitness due to inbreeding depression.

The effects of inbreeding depression were not incorporated into Zayed and Packer's (2005) population viability models, which showed the potential of high rates of extinction due to the production of diploid males in small bee populations. Given the theoretical expectations and empirical evidence, it is likely that inbreeding depression can interact synergistically with the sex determination load (Box I) to increase extinction risk over the already high values predicted by Zayed and Packer's (2005) models. Modelling the effects of inbreeding depression on population viability is possible following methods established for diploid organisms (Lacy, 1993; Brook et al., 2002b), although parametrizing such models would be difficult given lack of empirical data for bees. Future studies which attempt to quantify inbreeding depression in inbred but not match-mated bees (i.e. eliminating the confounding effects of the sex-determination load) will provide important empirical data needed for a formal examination of the effects of inbreeding depression on the viability of bee populations.

2.3. Haplodiploidy, genetic diversity, and bee declines

Genetic diversity is needed for populations to adapt to their changing environments (Fisher, 1930; Lynch and Walsh, 1998). The population's effective size, N_e (Box I), is an important determinant of standing levels of genetic diversity. Populations with small N_e experience stronger drift, and consequently maintain less genetic variation than those with large N_e . As a result, small populations are believed to have limited potential evolutionary responses to future changes in their environment (e.g. to novel pathogens, pesticides and contaminants, introduced species, habitat fragmentation, climate change, etc.) thereby increasing their long-term risk of extinction (reviewed by Frankham et al., 2002; Gaggiotti, 2003; Hedrick, 2004; Frankham, 2005). Since a haplodiploid population will have less gene copies compared to an equivalent diploid population, the former will have lower N_e (except when sex ratios are extremely female biased, see Crozier, 1976a). All other factors being equal – including mutation rates – a haplodiploid population is therefore expected to have lower levels of neutral genetic variation (Box I) than a diploid population. Complementary sex determination further reduces the already lower N_e of haplodiploids by reducing the number of females produced every generation (i.e. due to diploid male production), and biasing the secondary sex ratio in favor of haploid males when compared to an identical haplodiploid population without sl-CSD (Zayed, 2004). In addition to haplodiploidy and complementary sex determination, several other ecological attributes of bees contribute to lower N_e (Packer and Owen, 2001), of which the best known are eusociality and oligolecty. In eusocial bees, only a small proportion of the population is actually reproductive (i.e. queens and males) thereby reducing N_e by orders of magnitude over a comparable solitary population with the same census size (Pamilo and Crozier, 1997). Furthermore, the production of males by workers in some social species also serves to increase drift and lower genetic diversity (Owen, 1985). Oligolecty, or diet-specialization, has also been hypoth-

esized to reduce N_e by limiting both the population density and dispersal opportunities of specialist when compared to generalist bees (Packer et al., 2005). The hypothesized lower N_e of haplodiploid versus diploid insects, social versus solitary bees, and specialist versus generalist bees have been supported by comparative studies of genetic diversity. Haplodiploid insects – mostly hymenopterans – have less neutral genetic variation when compared to *Drosophila* (Hedrick and Parker, 1997) and Lepidopteran insects (Packer and Owen, 2001), while eusocial bees tend to exhibit lower levels of neutral genetic variation when compared to solitary bees (Packer and Owen, 2001). However, both of the previously mentioned meta-analyses do not correct for phylogeny (e.g. systematic differences between hymenopterans and lepidopterans, other than haplodiploidy, may be responsible for the observed differences), and their results should thus be interpreted with caution. Lower levels of neutral genetic variation in specialist versus generalist bees have been documented in several studies that correct for the confounding effects of phylogeny (Packer et al., 2005; Zayed et al., 2005).

Although there is some empirical support that haplodiploidy, eusociality, and oligolecty reduce N_e , this does not necessarily imply that the haplodiploid bees in general, and eusocial or specialist bees in particular, are more at risk of extinction due to their lowered ability to adapt to their changing environments. The evolutionary potential of a population is best predicted by measuring levels of additive genetic variance (Box I) in quantitative traits affecting fitness, a very difficult parameter to quantify in natural populations (Falconer and Mackay, 1996; Pfrender et al., 2000; Reed and Frankham, 2001; Gaggiotti, 2003; Hedrick, 2004). Neutral genetic variation often poorly reflects additive genetic variance mostly due to the effects of selection and differences in mutation rates (Pfrender et al., 2000; Reed and Frankham, 2001; Gaggiotti, 2003; Hedrick, 2004). For example, the mutation input for a polygenic trait should be larger than that observed at molecular markers. Further, variation at neutral markers is not expected to reflect variation in a quantitative trait where selection

– instead of drift – may be the primary evolutionary force. Therefore, lower neutral genetic variation in bees should not be taken as evidence that bees are more at risk of extinction due to reduced evolutionary potential when compared to other taxa.

Although there is no empirical evidence suggesting that losses of adaptive genetic diversity are contributing to the observed declines in bee populations, the threat is both conceivable and warrants further investigation. For example, pathogens and parasites have been linked to declines of both managed and native bees (Colla et al., 2006; Cox-Foster et al., 2007; Otterstatter and Thomson, 2008). In honey bees, there is a clear genetic basis for resistance to parasites (e.g. for resistance to *Varroa* mites, Harbo and Harris, 1999; Le Conte et al., 2007). Several quantitative trait loci (QTL) have been found to control hygienic behavior of worker honey bees (Lapidge et al., 2002) – a behavioral trait which limits parasite and pathogen loads in a colony. Similarly, several QTLs have been found to affect parasite infection intensity and general immune response in the bumble bee *Bombus terrestris* (Wilfert et al., 2007a, b). Therefore, it is plausible that loss of genetic variation at loci affecting pathogen / parasite resistance and general immune response may reduce a bee population's ability to cope with infections from novel pathogens or parasites. Future research is clearly needed to estimate levels of additive genetic variance in fitness-related traits and examine its consequences on population viability in natural bee populations. Experimental studies which attempt to directly estimate the effects of reduced adaptive diversity on population viability after controlling for other confounding factors (e.g. inbreeding depression, and complementary sex determination) are particularly needed (e.g. Vilas et al., 2006).

3. USE OF GENETICS IN BEE CONSERVATION MANAGEMENT

The application of molecular and population genetics to the field of conservation biology has provided a wealth of information on fundamental population parameters and

species characteristics relevant to wildlife conservation management. In this section, I provide a brief review of how molecular markers have been utilized to ascertain important population characteristics and processes in bees, and how conservation genetics can be used to help overcome pollinator decline.

3.1. Resolving taxonomic uncertainty

In order to implement conservation actions, knowledge about the taxonomic status of the targeted species is needed. It is obvious that taxonomic uncertainty due to the presence of cryptic species can hinder conservation efforts (Frankham et al., 2002). Molecular markers have been previously used to resolve cryptic bee species (e.g. Blanchetot and Packer, 1992; Carman and Packer, 1997; Danforth et al., 1998; Franck et al., 2004; Kuhlmann et al., 2007; Tavares et al., 2007; Murray et al., 2008). This is commonly achieved by observing high DNA sequence divergence, or large genetic distances (Box I), between species when contrasted against divergence/distance measures within species. For example, in a survey of genetic diversity in the morphologically distinct bee 'species' *Halictus ligatus* from eastern North America, Carman and Packer (1997) found fixed differences at more than 7 out of 34 allozyme loci (Box I) between populations in southern South Carolina and Florida when compared to all other populations, suggesting the presence of a cryptic species (*H. poeyi*). This was later confirmed by sequencing approximately 800 bp from several mtDNA genes from a few individuals from northern (*H. ligatus*) and southern (*H. poeyi*) populations: sequence divergence between *H. ligatus* and *H. poeyi* exceed 4% while intraspecific sequence divergence was very low (< 0.6%) (Danforth et al., 1998).

The application of molecular markers has helped in resolving taxonomic uncertainty of several endangered bees. For example, using a combination of allozymes, microsatellite (Box I) and RAPD markers, Tavares et al. (2007) found high levels of genetic differentiation between several geographic populations of the endangered stingless bee *Melipona rufigiventris*, indicating the presence of a cryptic

species which should be considered separately for conservation purposes. Similarly, Quezada-Euán et al. (2007) surveyed genetic diversity at several microsatellite markers and 678 bp of the mitochondrial gene COI in the endangered stingless bee *Melipona beecheii* and found high levels of genetic differentiation between populations in the Yucatan peninsula and Costa Rica. Quezada-Euán et al. (2007) recommended that movement of colonies between southern Mexico and Central America should be reconsidered given that the two regions may harbor two cryptic species.

The discovery of a nearly universal DNA barcode for animals (Hebert et al., 2003) is expected to greatly improve efforts to conserve bees. The DNA barcode represents a short (658 bp) sequence of the mitochondrial gene COI which shows high divergence between closely related species of most animal taxa, allowing for an efficient and nearly universal DNA identification system (Hebert et al., 2003; but see Moritz and Cicero, 2004). Conservation workers can quickly identify a targeted species by sequencing its DNA barcode and comparing it to a database of DNA barcodes from known taxa. COI has an established history of delineating closely related bee species (Danforth et al., 1998; Danforth, 1999; Dick et al., 2004; Quezada-Euán et al., 2007; Murray et al., 2008), with some rare exceptions (Kuhlmann et al., 2007). The application of DNA barcoding has already proved to be useful in resolving uncertainty in the taxonomically difficult *Lasioglossum* subgenus *Dialictus* (Gibbs, 2009), and a campaign to barcode the bees of the world has been initiated (see www.bee-bol.org).

3.2. Estimating species characteristics relevant to conservation

Neutral molecular markers have been utilized to great effect in estimating several critical and often unknown demographic characteristics of species targeted for conservation (Frankham et al., 2002; Hedrick, 2004). Because of their high variability, microsatellite markers (Box I) have high statistical power to estimate demographic parameters, and have thus emerged as the gold standard in population genetic surveys (Luikart and England,

1999). Indeed, the availability of microsatellite data has stimulated the development of novel statistical methods aimed at characterizing a species' demography (e.g. population structure, detection of first generation migrants and admixed individuals; testing for recent population size changes) (reviewed by Excoffier and Heckel, 2006). The growing availability of microsatellite markers combined with non-lethal techniques to sample DNA (Holehouse et al., 2003; Chaline et al., 2004) should open the door for population genetic studies of common, declining, and even endangered bees.

Population genetic surveys of natural bee populations have almost always detected biologically-significant levels of population structure. Packer and Owen (2001) reviewed allozyme-based studies and found that bees, along with other Hymenoptera, tend to have significantly higher estimates of genetic differentiation when compared to lepidopteran insects, suggesting that haplodiploidy promotes genetic differentiation, possibly through its effect on reducing effective population size. More recent studies using microsatellites have corroborated the earlier findings (e.g. Widmer and Schmid-Hempel, 1999; Danforth et al., 2003; Herrmann et al., 2007; Stow et al., 2007; Zayed and Packer, 2007), with some exceptions (Estoup et al., 1996; Beveridge and Simmons, 2006). Population genetic studies of endangered bumble bees also discovered significant levels of genetic differentiation (Darvill et al., 2006; Ellis et al., 2006), even at small geographic scales (< 10 km). The available evidence suggests that population genetic structure is a nearly universal feature of bee populations. This may be expected since nest-building and central place foraging can promote population subdivision by reducing gene flow.

Molecular markers have also been used to estimate colony densities, foraging ranges, and effective population sizes for several bee species. In a monoandrous haplodiploid species, sisters are expected to be 75% related to each other. If female bees are sampled as they forage, it is possible to detect full sisters based on their high degree of relatedness as inferred from genotyping a large number of hypervariable markers such as microsatellites

(Chapman et al., 2003). By estimating the number of colonies contributing foragers (i.e. sisters) at any particular site, and by making some assumptions regarding the number of colonies not observed, it is possible to estimate the number and density of nests at the sampled sites (Darvill et al., 2004). Also, the foraging range of a species can be estimated by examining the geographic distances separating foragers from the same colony (Chapman et al., 2003; Darvill et al., 2004). The above methods have been used to great effect in estimating nest densities and foraging ranges for common bumble bees (Chapman et al., 2003; Darvill et al., 2004; Knight et al., 2005). Sistership-based methods, as outlined above, can also be used to estimate the effective number of breeding individuals in a population (equals $1.5X$ the number of nests assuming monandry; Ellis et al., 2006).

Although more data are clearly needed, estimates of effective population sizes of bees tend to be surprisingly low. Zayed and Packer (2007), using changes in microsatellite allele frequencies over time (Wang, 2005), estimated current N_e to range from 200 to 1000 for several populations of the solitary sweat bee *Lasioglossum (Sphecodogastra) oenotherae*, a specialist on evening primroses. Using a sistership-based method, Ellis et al. (2006) estimated the breeding population size (i.e. which provides an upper limit for N_e) of the endangered bumble bee *Bombus sylvarum* to range 21 to 72. Low historical N_e , inferred from allozymes, have also been observed for the common sweat bee *Halictus poeyi* (Zayed and Packer, 2001), and the declining orchid bee *Euglossa imperialis* (Zayed et al., 2004). More estimates of N_e for solitary and social bees are badly needed before any broad conclusions can be made, but the available data seem to suggest that bees persist in somewhat isolated populations with comparatively small effective sizes. Low N_e and limited gene flow can exacerbate both genetic and demographic threats to population viability (see Sect. 2), and conservation actions developed for bees must be mindful of these characteristics.

Although population genetic surveys are very insightful on their own, they become extremely illuminating when combined

with ecological studies, as the recent work by Herrmann et al. (2007) demonstrates. Herrmann et al. (2007) sampled workers of the common bumble bee *Bombus pascuorum* from 13 sites that differed in land-use types in an agricultural landscape in Germany, and the sampled workers were genotyped at 8 microsatellite loci. Herrmann et al. (2007) were able to estimate important demographic and genetic parameters for each site (e.g. number of colonies, colony density, relative colony size, estimates of genetic differentiation, and inbreeding coefficients). The authors then used generalized linear models to examine the relationship between demographic parameters, genetic parameters, and land-use characteristics. Herrmann et al. (2007) found that the proportion of mass flowering crops positively affected bumble bee abundance, and that positive inbreeding coefficients (i.e. higher frequency of homozygotes than expected assuming Hardy-Weinberg equilibrium) had a negative effect on colony size. The latter result represents strong evidence that increased homozygosity adversely affects fitness of bee populations, and this effect is most likely mediated through the production of diploid males (Sect. 2.1) and possibly inbreeding depression (Sect. 2.2). Herrmann et al.'s (2007) work clearly demonstrates that the integration of population genetic and ecological studies is likely to yield findings of great significance for examining the ecological and genetic processes which underpin declines in bee populations.

3.3. Detecting declining bee species using genetic methods

The first step to conserving a species involves demonstrating that it is on the decline. Biodiversity data (presence/absence of species, or population census data) have been mostly used for detecting declines in bee populations (Roubik, 2001; Williams et al., 2001; Biesmeijer et al., 2006; Grixiti and Packer, 2006; Colla and Packer, 2008; Grixiti et al., 2009). However, natural variation in the abundance of bees over space and time complicates the detection of population decline (Roubik,

2001; Williams et al., 2001). Population genetic data, on the other hand, reflect both the current state of the population as well as its history, making such data more resistant to the temporal and spatial variation found in ecological datasets. There are several genetic approaches to detecting recent reductions in effective population sizes. One approach involves detecting population bottlenecks. Bottleneck tests rely on the fact that reductions in population size create some disparity among the different ways of quantifying genetic diversity (Cornuet and Luikart, 1996; Excoffier and Heckel, 2006). For example, following a bottleneck, allelic richness is reduced faster when compared to heterozygosity: An elevated heterozygosity compared to that expected based on allelic richness can indicate recent reductions in population size (Cornuet and Luikart, 1996). Applying this method to microsatellite data, bottlenecks were detected in several populations of two endangered and declining bumble bee species (Darvill et al., 2006; Ellis et al., 2006). Using simulation-based approaches, it is also possible to estimate past population sizes, the magnitude of change in population size, and the relative timing of bottlenecks (Excoffier and Heckel, 2006), providing potential clues regarding the causes of the declines.

Another method of detecting declines of bee populations involves using frequencies of diploid males (Zayed et al., 2004). The frequency of diploid males is a negative function of effective population size (Fig. 2; Sect. 2.1), and declining populations are expected to produce higher frequencies of diploid males. Therefore, increased levels of diploid male production can theoretically indicate bee population declines (Zayed et al., 2004). Genetic monitoring of bee populations can be undertaken by first establishing baseline data of diploid male production in populations of interest, followed by routine sampling of males – preferably through non-lethal DNA extraction protocols – to measure changes in that parameter over time. This, of course, assumes that bees are not able to adaptively avoid producing diploid males as the number of sex determining alleles declines (see Sect. 2.1). Also, to ensure that frequencies of

diploid male production estimated from sampling adults are unbiased, preliminary studies of diploid male survival will be needed before field monitoring programs are established.

The recent characterization of the sex-determination locus in the honey bee (Beye et al., 2003; Hasselmann et al., 2008) can also aid in developing ways to directly assess the number of sex-determining alleles in a population. In principal, PCR can be used to amplify the sex-determination locus in samples of bees, followed by cloning and sequencing of the amplified PCR products to examine sequence diversity at that locus. This approach has one major advantage over detecting population declines through frequencies of diploid males, since the latter requires that diploid males be sampled in an unbiased way, which may be difficult if they have low survivorship. However, it remains to be demonstrated that the sex-determination gene characterized in the honey bee is sufficiently well-conserved to allow for constructing universal PCR primers for routine amplification of the locus in other bee taxa. We also still lack knowledge on how alleles are ‘functionally’ encoded at the sex-determination locus, and thus the use of sequence diversity as a surrogate for allelic diversity at the sex determination locus may not be appropriate. Further studies are needed to examine the utility of using PCR-based methods to quantify allelic diversity at the sex determining gene, and its potential use in detecting declines in bee populations.

The above approaches for genetically detecting population declines can prove very useful in bee conservation management, however, they can sometimes be undermined by demographic history. For example, if a population experienced a drastic bottleneck in the past, but has rapidly recovered since, both of the proposed methods would suggest that the population is in decline. For example, the solitary sweat bee *Lasioglossum leucozonium* and the bumble bee *Bombus terrestris* exhibit significant signs of recent bottlenecks and high frequencies of diploid male production even though they are actually increasing in their invasive ranges in North America (Zayed et al., 2007) and Tasmania (Schmid-Hempel et al., 2007) respectively. However, in both cases,

the genetic data also indicated that the studied populations were invasive. The risk of misdiagnosis can further be reduced if the genetic data are interpreted in light of the ecology and natural history of the species in question.

Finally, the above mentioned approaches can be greatly leveraged by genetic analyses of ‘historic’ specimens from museum collections. Population genetic studies using specimens from museum collections can be used to contrast historical levels of genetic diversity and differentiation with present-day levels (Wandeler et al., 2007). A recent study has demonstrated that old bumble bee specimens (as old as 100 years) can be genotyped at microsatellite loci using PCR (Strange et al., 2009). Population genetic analyses of museum specimens have also proved useful in quantifying demographic changes in several common and declining Midwestern bumble bee species (Lozier and Cameron, 2009).

3.4. The next frontier of conservation biology: Genomics and bee conservation

The emerging field of Genomics has the potential to contribute considerably to the conservation management of wildlife populations. Three particular developments have clear utility in bee conservation management: (1) New and cost-effective sequencing technologies; (2) Global gene expression profiling using microarrays; and (3) Population genomic studies using Single Nucleotide Polymorphism (SNP) markers (Box I). New sequencing technologies (Shendure et al., 2004; Margulies et al., 2005; Strausberg et al., 2008) can generate large amounts of data at a fraction of the cost of traditional Sanger sequencing, although the sequences have shorter read length. These technologies can be used to re-sequence the known genomes of model organisms, or for *de novo* sequencing of the genomes or transcriptomes of non-model organisms. This is an important first step for the development of platforms for measuring global gene expression, and for developing high-throughput genotyping platforms for population genomic analysis (see below). New sequencing technologies

can also be used in ‘forensic’ applications. For example, Cox-Foster et al. (2007) used pyrosequencing technology (Margulies et al., 2005; 454 Life Sciences) to sequence RNA from honey bees (*Apis mellifera*) and royal jelly found in healthy colonies, and colonies afflicted with colony collapse disorder (CCD). By matching the generated sequences to public sequence databases, the study found that the presences of several pathogens, including Israeli acute paralysis virus, were correlated with CCD. Although Cox-Foster et al.’s (2007) meta-genomic study is not statistically appropriate for establishing cause and effect, it provides important knowledge which can be used to develop and test causal hypotheses regarding the role of pathogens in CCD of honey bees.

Global analysis of gene expression, through the use of microarrays, has provided invaluable insights into nearly all fields of biology (Schena et al., 1995; Brown and Botstein, 1999; Gibson, 2002; Neumann and Galvez, 2002; Cowell and Hawthorn, 2007; van ’t Veer and Bernards, 2008), and has great potential for bee conservation management. After a species’ transcriptome is characterized through complete or partial sequencing, short DNA probes for the discovered transcripts can be synthesized on glass slides called microarrays. The relative mRNA abundance for all genes in the genome – at a specific tissue at a specific time – can thus be measured through hybridization of fluorescently-labelled transcripts isolated from the tissues of interest with the probes on the microarray.

Microarray experiments are very effective in detecting gene expression differences across experimental groups (e.g. healthy or diseased tissue), and are thus naturally suited to forensic and diagnostic applications. For example, microarray experiments on healthy and cancerous organs have indicated many differences in gene expression levels between the two (Cowell and Hawthorn, 2007; Nevins and Potti, 2007). Knowledge of differential gene expression has been used to better understand the molecular processes underlying cancer by identifying potentially causal candidate genes, in addition to enabling more accurate diagnoses through gene expression profiles (e.g.

over expression of certain genes can be used to identify cancer) (Wadlow and Ramaswamy, 2005). The same principles can be applied to the field of wildlife conservation. Microarray experiments comparing gene expression profiles between individuals from healthy versus declining populations can be used to predict the 'health' of a population, and to test hypotheses regarding the causes of declines. For example, differential expression of genes involved in activating the innate immune system in a declining population may suggest that pathogen infections and not some other factor (e.g. pesticides) is associated with the decline. Microarrays have been developed for the honey bee *Apis mellifera* (Whitfield et al., 2002), and have recently been used to examine differences in gene expression associated with *Varroa* mite parasitism in both susceptible and tolerant colonies (Navajas et al., 2008). Navajas et al. (2008) found that *Varroa* parasitism affects the expression of several genes associated with immune function and neural development. Workers from tolerant colonies showed differential expression for several genes affecting neural sensitivity and olfaction, suggesting that behavior underlies tolerance to *Varroa*. Navajas et al.'s (2008) study demonstrates the utility of microarrays in identifying genes associated with particular parasites/pathogens in bees, which can then lead to the development of specific management tools. Although genomic tools are currently only available for the honey bee *Apis mellifera*, there are plans to generate genomic resources for other bees. For example, Dr. Gene Robinson (University of Illinois at Urbana Champaign) is leading efforts to sequence partial brain transcriptomes of 12 species spanning the entire phylogeny of bees (G.E. Robinson, unpubl. data). The availability of the honey bee genome sequence (The Honeybee Genome Sequencing Consortium, 2006), combined with the expected development of genomic resources for other bees, will lay the ground work for developing microarray technology that can be used for investigating the causes of bee declines in both model and non-model species.

Finally, population genomic studies using SNP markers can be used for a variety of

applications with relevance to conservation management (Morin et al., 2004). Following the discovery of SNPs through sequencing, customized SNP chips can be created to genotype hundreds to thousands of SNPs, allowing for the characterization of genetic diversity across the genome in natural populations (Twyman, 2004; Kim and Misra, 2007). The data can be used to conduct traditional population genetic analyses (see Sects. 3.1 and 3.2), as well as to discover areas of the genome experiencing selection (Morin et al., 2004; Nielsen, 2005; Biswas and Akey, 2006; Sabeti et al., 2006; Nielsen et al., 2007). A recent survey of SNP diversity in native and introduced *Apis mellifera* populations clearly demonstrates the utility of the approach (Whitfield et al., 2006; Zayed and Whitfield, 2008; De la Rúa et al., 2009). Whitfield et al. (2006) used a panel of ~1100 SNPs to genotype ~350 honeybees from across their native and introduced ranges. The results supported earlier findings of four major geographic populations of the honey bee, but uncovered that the honey bee actually originated in Africa, and not Asia as previously believed. Zayed and Whitfield (2008) then interrogated the dataset for signatures of selection based on differences in levels of genetic differentiation measured by SNPs in functional versus nonfunctional and presumably neutral regions of the genome. They found strong signatures of positive selection and adaptive evolution associated with the ancient and recent-invasive expansion of honey bees out of Africa into West Europe and the New World respectively. The ability of SNP datasets to both estimate demographic parameters and uncover instances of adaptive evolution will undoubtedly move conservation biology a step closer to understanding the genetic basis underlying fitness traits in natural populations (Mitchell-Olds et al., 2007; Ellegren and Sheldon, 2008), and possibly provide the means to quantify the evolutionary potential of small endangered populations (Morin et al., 2004).

4. CONCLUSIONS

Bees are indispensable components of terrestrial ecosystems and their conservation is

essential for both ecological and economic reasons. Efforts to conserve declining bee populations can only be as effective as our knowledge of their biology, and the causes contributing to their declines. The application of genetics to bee conservation biology can be of great use in aiding bee conservation management, and in identifying genetic threats to the short term viability of bee populations. Population genetic surveys can be, and have indeed been used, to provide knowledge about several important parameters of relevance to bee conservation biology, including population structure, gene flow, effective population sizes, colony densities, and foraging ranges. Genetic approaches can also be used for rapid species identification, for resolving taxonomic conflict, and for indicating bee populations in decline. They also provide more robust and timely estimates of population size in comparison to the vagaries of census methods. As more genetic and genomic resources are developed for bees, it will become progressively easier and cheaper to gather population genetic data from both common and endangered bee species for comparative analyses and conservation studies. Integrating population genetic surveys with ecological studies, although rarely undertaken, can provide greater insight into bee conservation biology than either approach practiced alone.

The production of inviable or sterile diploid males, a necessary by-product of complementary sex determination, is a large threat to the short-term viability of small bee populations, as indicated both by theory and mostly indirect empirical data. Inbreeding depression, caused by dominance or overdominance, although expected to be lower in haplodiploids when compared to diploids, should still contribute to reduced fitness and reduced viability of small bee populations. Inbreeding depression is expected to interact synergistically with diploid male production causing greater rates of extinction than the already high rates caused by the latter. These genetic factors will also interact synergistically with extrinsic factors causing declines in bee populations. The above mentioned genetic threats to population viability imply that: (1) Bees are highly susceptible to extinction in small

and/or isolated populations. (2) When subjected to an extrinsic factor causing decline, bee populations will do so at a faster rate than expected based on the direct effect of the extrinsic factor. (3) Bee populations should recover at a slower rate than expected following the removal of extrinsic factors causing declines. Bee populations targeted for conservation should be managed to reduce frequencies of diploid male production, and to a lesser extent, inbreeding depression.

ACKNOWLEDGEMENTS

I am most grateful to Laurence Packer for motivating and guiding my research on bee conservation genetics. I would like to thank Laurence Packer, Rob Paxton, Richard Frankham, Jennifer Grixti, and Jeff Lozier for providing helpful comments on the manuscript. I also thank Jennifer Grixti, and Jamie Strange for providing copies of their manuscript slated for publication, and to Gene Robinson, Jason Gibbs, Laurence Packer, and Jeff Lozier for allowing me to refer to their unpublished research. Finally, I wish to thank Jason Alexander Zayed for providing inspiration and a stimulating environment to write this review. My work is supported by a Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship, and a postdoctoral fellowship from the Institute for Genomic Biology, University of Illinois at Urbana-Champaign.

Génétique des abeilles et conservation des espèces.

Apoidea / haploïdiploïdie / dépression consanguinité / mâle diploïde / extinction / détermination sexe complémentaire / abeille

Zusammenfassung – Bienengenetik und Artenschutz. In dieser Arbeit betrachten wir, welche Rolle die Genetik im Rückgang der Bienenpopulationen spielt und wie die Werkzeuge der Genetik in Bienenschutzprojekten eingesetzt werden können. Genetische Marker haben sich als höchst nützlich erwiesen in der Bestimmung wichtiger demographischer Parameter von Bienenpopulationen (z.B. Populationsgrößen, Dichte, Vernetzung, Ausbreitungsraten und Sammelradien), sowie zur Klärung taxonomischer Probleme und zur Erkennung von

Rückgängen in Populationen. Neuere Fortschritte in der Bienengenomik sollten ebenfalls einen Beitrag zur Erleichterung von Untersuchungen über die Ursachen von Rückgängen in Bienenpopulationen leisten. Die Integration der Kenntnisse zur Genetik und Genomik der Bienen mit denen ihrer Ökologie sollte daher deutliche Verbesserungen in Bienen-schutzprogrammen zur Folge haben. Ein wichtiger Faktor, der kleine Bienenpopulationen bedroht, ist die durch Homozygotie am Geschlechtslokus ausgelöste Produktion nicht lebensfähiger, steriler diploider Männchen anstelle von Weibchen (Abb. 1). Das Vorkommen diploider Männchen ist für mindestens 27 Arten beschrieben (Tab. 1) und die Frequenz dieser Männchen nimmt in kleinen Populationen durch Homozygotie am Geschlechtsbestimmungs-lokus bedingt durch genetische Drift zu (Abb. 2). Simulationsstudien lassen erwarten, dass die Produktion diploider Männchen die Populationswachstumsraten vor allem bei solitären Bienen reduzieren kann, die eine niedrige Fekundität in ihrer Gesamt-lebenszeit aufweisen. Das Problem der Produktion diploider Männchen geht so Hand in Hand mit negativen Umweltfaktoren und führt zu einem schnelleren Rückgang und einer langsameren Erholung von Populationen (Abb. 3). Sie spielt damit eine wichtige Rolle im weltweiten Rückgang von Bienen.

Ausser der Produktion diploider Männchen könnte auch eine Inzuchtdepression das Überleben kleiner Bienenpopulationen gefährden. Obwohl zu erwarten wäre, dass die Haplodiploidie die genetische Bürde bei Bienen reduziert, zeigen sowohl theoretische Studien als auch empirische Befunde, dass selbst haplodiploide Organismen eine erhebliche Inzuchtdepression durchlaufen können. Schutzprogramme für gefährdete und zurückgehende Bienenpopulationen sollten daher bestrebt sein, die Produktion diploider Männchen und Inzucht zu reduzieren.

Haplodiploidie / Komplementäre Geschlechts-determinierung / Inzuchtdepression / diploide Männchen / Artensterben

BOX I: GLOSSARY OF GENETIC TERMS

Additive genetic variance: The contribution of genetic variance in a quantitative trait due to the effects of substituting one allele for another at a locus.

Allozymes: Alternative forms of a particular protein as visualized on a gel, mostly resulting from genetic variation at non-synonymous sites in a gene's protein coding sequence.

Single locus complementary sex determination: Sex is determined by genotype at a single locus: heterozygotes, homozygotes and hemizygotes develop into diploid females, diploid males, and haploid males respectively.

Diploid species: A species where both sexes have two copies of each autosomal chromosome.

Effective population size, N_e : The size of an 'ideal' population (a random mating population of constant size with Poisson variation in family sizes) that would have the same genetic parameters (e.g. genetic drift) as the actual population under study.

Evolutionary potential: The ability of populations to adapt to future changes in their environments.

Frequency dependent selection: A form of natural selection where the relative fitness of genotypes is a function of their frequency. Negative and positive frequency dependent selection denotes higher fitness of, and selection for, rare or common genotypes respectively. The sex determination locus in the Hymenoptera is under **negative frequency dependent selection** since the fitness of individuals carrying rare sex-determining alleles is higher as they are less likely to produce diploid males.

Genetic distance and differentiation: Parameters that measure the extent of genetic differences between populations, usually over space. Populations with similar gene pools (e.g. similar allele frequencies) have smaller genetic distances and levels of differentiation than populations with different gene pools.

Genetic drift: Changes in allele frequencies due to the effects of random sampling of gametes in finite populations. Genetic drift is stronger in smaller populations, and results in reduced genetic diversity.

Genetic load: The reduction in fitness of a population from the maximum possible due to the deleterious effects (i.e. load) of alleles. The genetic load can be due to the deleterious effects of mutations (i.e. mutational load) or due to lower fitness of homozygous genotypes relative to heterozygous genotypes (i.e. balance load).

Sex determination load: The reduction of fitness of a haplodiploid population from the

maximum possible due to the deleterious effects of homozygosity at the hymenopteran complementary sex determination locus.

Haplodiploid species: A species where females are diploid (i.e. have two copies of each chromosome), and males are haploid (i.e. have one copy of each chromosome).

Inbreeding: Several phenomena leading to mating between individuals related by descent, and resulting in increased homozygosity. Inbreeding in a mating-system sense, known as **systematic inbreeding**, results when relatedness between mates are higher than the average relatedness of mates chosen at random from the same population. **Inbreeding by drift**, or **panmictic inbreeding**, results from the increasing probability of relatedness among randomly chosen mates in a finite population over time.

Inbreeding depression: Reduction in the mean fitness of a trait due to inbreeding. Attributed to the increased expression of recessive or partially recessive deleterious alleles through increased homozygosity (**Dominance or Partial dominance hypothesis**), or to the reduction in the frequency of fitter heterozygous genotypes (**Overdominance hypothesis**).

Matched mating: A mating between a male and female that share a sex-determining allele in common. Half of the diploid progeny produced by a matched-mated female will be homozygous at the sex-determination locus, and develop into diploid males.

Microsatellite: A sequence of DNA composed of short (2-6 bases) tandem repeats (e.g. CACACACACA). Mutation at microsatellite loci results in alleles with different repeat numbers.

Neutral genetic variation: Genetic variation that does not affect fitness.

Single nucleotide polymorphism, SNP: A specific position in a DNA sequence which contains alternative bases.

REFERENCES

- Adams J., Rothman E.D., Kerr W.E., Paulino Z.L. (1977) Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*, *Genetics* 86, 583–596.
- Agoze M.E., Drezen J.M., Renalt S., Preiquet G. (1994) Analysis of the reproductive potential of diploid males in the wasp *Diadromus pulchellus* (Hymenoptera: Ichneumonidae), *Bull. Entomol. Res.* 84, 213–218.
- Allen-Wardell G., Bernhardt P., Bitner R., Burquez A., Buchmann S.L., Cane J.H., Cox P.A., Dalton V., Feinsinger P., Ingram M., Inouye D.W., Jones C.E., Kennedy K., Kevan P.G., Koopowitz H., Medellin R., Medellin-Morales S., Nabhan G.P. (1998) The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields, *Conserv. Biol.* 12, 8–17.
- Antolin M.F. (1999) A genetic perspective on mating systems and sex ratios of parasitoid wasps, *Res. Popul. Ecol.* 41, 29–37.
- Armbruster P., Reed D.H. (2005) Inbreeding depression in benign and stressful environments, *Heredity* 95, 235–242.
- Ayabe T., Hoshiba H., Ono M. (2004) Cytological evidence for triploid males and females in the bumblebee, *Bombus terrestris*, *Chromosome Res.* 12, 215–223.
- Beveridge M., Simmons L.W. (2006) Panmixia: an example from Dawson's burrowing bee (*Amegilla dawsoni*) (Hymenoptera: Anthophorini), *Mol. Ecol.* 15, 951–957.
- Beveridge M., Simmons L.W., Alcock J. (2006) Genetic breeding system and investment patterns within nests of Dawson's burrowing bee (*Amegilla dawsoni*) (Hymenoptera: Anthophorini), *Mol. Ecol.* 15, 3459–3467.
- Beye M., Hasselmann M., Fondrk M.K., Page R.E., Omholt S.W. (2003) The gene *csd* is the primary signal for sexual development in the honey bee and encodes a new SR-type protein, *Cell* 114, 419–429.
- Bienefeld K., Reinhardt F., Pirschner F. (1989) Inbreeding effects of queen and workers on colony traits in the honey bee, *Apidologie* 20, 439–450.
- Biesmeijer J.C., Roberts S.P.M., Reemer M., Ohlemuller R., Edwards M., Peeters T., Schaffers A.P., Potts S.G., Kleukers R., Thomas C.D., Settele J., Kunin W.E. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands, *Science* 313, 351–354.
- Biswas S., Akey J.M. (2006) Genomic insights into positive selection, *Trends Genet.* 22, 437–446.
- Blanchetot A., Packer L. (1992) Genetic variability in the social bee *Lasiosglossum marginatum* and a cryptic underscribed sibling species, as detected by DNA fingerprinting and allozyme electrophoresis, *Insect Mol. Biol.* 1, 89–97.

- Brook B.W., Burgman M.A., Akçakaya H.R., O'Grady J.J., Frankham R. (2002a) Critiques of PVA ask the wrong questions: throwing the heuristic baby out with the numerical bath water, *Conserv. Biol.* 16, 262–263.
- Brook B.W., Tonkyn D.W., O'Grady J.J., Frankham R. (2002b) Contribution of inbreeding to extinction risk in threatened species, *Conserv. Ecol.* 6, 16.
- Brown P.O., Botstein D. (1999) Exploring the new world of the genome with DNA microarrays, *Nat. Genet.* 21, 33–37.
- Byrne A., Fitzpatrick Ú. (2009) Bee conservation policy at the global, regional and national levels, *Apidologie* 40, 194–210.
- Camargo C.A. (1979) Sex determination in bees. XI. Production of diploid males and sex determination in *Melipona quadrifasciata*, *J. Apic. Res.* 18, 77–84.
- Cameron E.C., Franck P., Oldroyd B.P. (2004) Genetic structure of nest aggregations and drone congregations of the southeast Asian stingless bee *Trigona collina*, *Mol. Ecol.* 13, 2357–2364.
- Cane J.H. (2001) Habitat fragmentation and native bees: a premature verdict? *Conserv. Ecol.* 5, 3.
- Cane J.H., Tepedino V.J. (2001) Causes and extent of declines among native North American invertebrate pollinators: detection, evidence, and consequences, *Conserv. Ecol.* 5, 1.
- Carman G.M., Packer L. (1997) A cryptic species allied to *Halictus ligatus* Say (Hymenoptera; Halictidae) detected by allozyme electrophoresis, *J. Kans. Entomol. Soc.* 69, 168–176.
- Carvalho G.A. (2001) The number of sex alleles (CSD) in a bee population and its practical importance (Hymenoptera: Apidae), *J. Hymenoptera Res.* 10, 10–15.
- Chaline N., Ratnieks F.L.W., Raine N.E., Badcock N.S., Burke T. (2004) Non-lethal sampling of honey bee, *Apis mellifera*, DNA using wing tips, *Apidologie* 35, 311–318.
- Chapman R.E., Wang J., Bourke A.F.G. (2003) Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators, *Mol. Ecol.* 12, 2801–2808.
- Charlesworth B., Charlesworth D. (1999) The genetic basis of inbreeding depression, *Genet. Res.* 74, 329–340.
- Colla S.R., Packer L. (2008) Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson, *Biodivers. Conserv.* 17, 1379–1391.
- Colla S.R., Otterstatter M.C., Gegear R.J., Thomson J.D. (2006) Plight of the bumblebee: Pathogen spillover from commercial to wild populations, *Biol. Conserv.* 129, 461–467.
- Cook J.M. (1993) Sex determination in the Hymenoptera: a review of models and evidence, *Heredity* 71, 421–435.
- Cook J.M., Crozier R.H. (1995) Sex determination and population biology of the Hymenoptera, *Trends Ecol. Evol.* 10, 281–286.
- Cornuet J.M. (1980) Rapid estimation of the number of sex alleles in panmictic honeybee populations, *J. Apic. Res.* 19, 3–5.
- Cornuet J.M., Luikart G. (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data, *Genetics* 144, 2001–2014.
- Cowan D.P., Stahlhut J.K. (2004) Functionally reproductive diploid and haploid males in an inbreeding hymenopteran with complementary sex determination, *Proc. Natl Acad. Sci. USA* 101, 10374–10379.
- Cowell J.K., Hawthorn L. (2007) The application of microarray technology to the analysis of the cancer genome, *Curr. Mol. Med.* 7, 103–120.
- Cox-Foster D.L., Conlan S., Holmes E.C., Palacios G., Evans J.D., Moran N.A., Quan P.-L., Briese T., Hornig M., Geiser D.M., Martinson V., vanEngelsdorp D., Kalkstein A.L., Drysdale A., Hui J., Zhai J., Cui L., Hutchison S.K., Simons J.F., Egholm M., Pettis J.S., Lipkin W.I. (2007) A Metagenomic Survey of Microbes in Honey Bee Colony Collapse Disorder, *Science* 318, 283–287.
- Crnokrak P., Barrett S.C. (2002) Perspective: purging the genetic load: a review of the experimental evidence, *Evolution* 12, 2347–2358.
- Crozier R.H. (1976a) Counter-intuitive property of effective population size, *Nature* 262, 384.
- Crozier R.H. (1976b) Why Male-Haploid and Sex-Linked Genetic Systems Seem to Have Unusually Sex-Limited Mutational Genetic Loads, *Evolution* 30, 623–624.
- Crozier R.H. (1977) Evolutionary genetics of the hymenoptera, *Annu. Rev. Entomol.* 22, 263–288.
- Crozier R.H. (1985) Adaptive consequences of male-haploidy, in: Helle W., Sabelis M.W. (Eds.), *Spider Mites: their biology, natural enemies and control*, Elsevier Science Publisher B.V. Amsterdam, pp. 201–222.
- Danforth B.N. (1999) Phylogeny of the bee genus *Lasioglossum* (Hymenoptera: Halictidae) based on mitochondrial COI sequence data, *Syst. Entomol.* 24, 377–393.
- Danforth B.N., Mitchell P.L., Packer L. (1998) Mitochondrial DNA differentiation between two cryptic *Halictus* (Hymenoptera: Halictidae) species, *Ann. Entomol. Soc. Am.* 91, 387–391.
- Danforth B.N., Ji S., Ballard L.J. (2003) Gene flow and population structure in an oligolectic desert bee, *Macrotera (Macroteropsis) portalis*

- (Hymenoptera: Andrenidae), *J. Kans. Entomol. Soc.* 76, 221–235.
- Danks H.V. (1971) Nest mortality factors in stem-nesting aculeate Hymenoptera, *J. Anim. Ecol.* 40, 79–82.
- Darvill B., Knight M.E., Goulson D. (2004) Use of genetic markers to quantify bumblebee foraging range and nest density, *Oikos* 107, 471–478.
- Darvill B., Ellis J.S., Lye G.C., Goulson D. (2006) Population structure and inbreeding in a rare and declining bumblebee, *Bombus muscorum* (Hymenoptera: Apidae), *Mol. Ecol.* 15, 601–611.
- De Boer J.G., Ode P.J., Vet L.E.M., Whitfield J., Heimpel G.E. (2007) Complementary sex determination in the parasitoid wasp *Cotesia vestalis* (*C. plutellae*), *J. Evol. Biol.* 20, 340–348.
- De la Rúa P., Jaffé R., Dall’Olio R., Muñoz I., Serrano J. (2009) Biodiversity, conservation and current threats to European honeybees, *Apidologie* 40, 263–284.
- Dias B.S.F., Raw A., Imperatriz-Fonseca V.L. (1999) International pollinators initiative: The São Paulo declaration on pollinators., Brazilian Ministry of the Environment, Brasilia. [online] <http://www.cbd.int/doc/case-studies/agr/cs-agr-pollinator-rpt.pdf> (accessed on 2 February 2009).
- Dick C.W., Roubik D.W., Gruber K.F., Bermingham E. (2004) Long-distance gene flow and cross-Andean dispersal of lowland rainforest bees (Apidae: Euglossini) revealed by comparative mitochondrial DNA phylogeography, *Mol. Ecol.* 13, 3775–3785.
- Duchateau M.J., Hoshiba H., Velthuis H.H.W. (1994) Diploid males in the bumblebee *Bombus terrestris*: sex determination, sex alleles and viability, *Entomol. Exp. Appl.* 71, 263–269.
- Eickwort G.C., Ginsberg H.S. (1980) Foraging and mating behavior in Apoidea, *Annu. Rev. Entomol.* 25, 421–446.
- Ellegren H., Sheldon B.C. (2008) Genetic basis of fitness differences in natural populations, *Nature* 452, 169–175.
- Ellis J.S., Knight M.E., Darvill B., Goulson D. (2006) Extremely low effective population sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species, *Bombus sylvarum* (Hymenoptera: Apidae), *Mol. Ecol.* 15, 4375–4386.
- Else G., Felton J., Stubbs A. (1978) The Conservation of Bees and Wasps, Nature Conservancy Council, Peterborough.
- Estoup A., Scholl A., Pouvreau A., Solignac M. (1995) Monoandry and polyandry in bumble bees (Hymenoptera: Bombinae) as evidenced by highly variable microsatellites, *Mol. Ecol.* 4, 89–94.
- Estoup A., Solignac M., Cornuet J.M., Goudet J., Scholl A. (1996) Genetic differentiation of continental and island populations of *Bombus terrestris* (Hymenoptera: Apidae) in Europe, *Mol. Ecol.* 5, 19–31.
- Excoffier L., Heckel G. (2006) Computer programs for population genetics data analysis: a survival guide, *Nat. Rev. Genet.* 7, 745–758.
- Falconer D.S., Mackay T.F.C. (1996) Introduction to Quantitative Genetics, Longman, Essex, UK.
- Fisher R.A. (1930) The Genetic Theory of Natural Selection, Dover, New York.
- Fitzpatrick U., Murray M.G., Paxton R.J., Breen J., Cotton D., Santorum V., Brown M.J.F. (2006) Rarity and decline in bumblebees - A test of causes and correlates in the Irish fauna, *Biol. Conserv.* 136, 185–194.
- Fontaine C., Dajoz I., Meriguet J., Loreau M. (2006) Functional Diversity of Plant-Pollinator Interaction Webs Enhances the Persistence of Plant Communities, *PLoS Biol.* 4, e1.
- Franck P., Cameron E., Good G., Rasplus J.Y., Oldroyd B.P. (2004) Nest architecture and genetic differentiation in a species complex of Australian stingless bees, *Mol. Ecol.* 13, 2317–2331.
- Frankham R. (1995a) Effective population size/adult population size ratios in wildlife: a review, *Genet. Res.* 66, 95–107.
- Frankham R. (1995b) Inbreeding and extinction: a threshold effect, *Conserv. Biol.* 9, 792–799.
- Frankham R. (1998) Inbreeding and extinction: island populations, *Conserv. Biol.* 12, 665–675.
- Frankham R. (2005) Genetics and extinction, *Biol. Conserv.* 126, 131–140.
- Frankham R., Ballou J.D., Briscoe D.A. (2002) Introduction to conservation genetics, Cambridge University Press, Cambridge.
- Franzén M., Larsson M. (2007) Pollen harvesting and reproductive rates in specialized solitary bees, *Ann. Zool. Fenn.* 44, 405–414.
- Gadau J., Gerloff C.U., Krüger N., Chan H., Schmid-Hempel P., Wile A., Page R.E.J. (2001) A linkage analysis of sex determination in *Bombus terrestris* (L.) (Hymenoptera: Apidae), *J. Hered.* 87, 234–242.
- Gaggiotti O.E. (2003) Genetic threats to population persistence, *Ann. Zool. Fenn.* 40, 155–168.
- Gerloff C.U., Schmid-Hempel P. (2005) Inbreeding depression and family variation in a social insect, *Bombus terrestris* (Hymenoptera: Apidae), *Oikos* 111, 67–80.
- Gibbs J. (2009) Integrative taxonomy identifies new (and old) species in the *Lasioglossum* (*Dialictus*) *tegulare* (Robertson) species group (Hymenoptera, Halictidae), *Zootaxa* 2032, 1–38.
- Gibson G. (2002) Microarrays in ecology and evolution: a preview, *Mol. Ecol.* 11, 17–24.

- Gilpin M.E. (1991) The genetic effective size of a metapopulation, *Biol. J. Linn. Soc. Lond.* 42, 165–175.
- Glémin S. (2003) How are deleterious mutations purged? Drift versus nonrandom mating, *Evolution* 57, 2678–2687.
- Goulson D., Lye G.C., Darvil B. (2008) Decline and conservation of bumble bees, *Annu. Rev. Entomol.* 53, 191–208.
- Green C.L., Oldroyd B.P. (2002) Queen mating frequency and maternity of males in the stingless bee *Trigona carbonaria* Smith, *Insectes Soc.* 49, 196–202.
- Grixti J.C., Packer L. (2006) Changes in the bee fauna (Hymenoptera: Apoidea) of an old field site in southern Ontario, revisited after 34 years, *Can. Entomol.* 138, 147–164.
- Grixti J.C., Wong L.T., Cameron S.A., Favret C. (2009) Decline of bumble bees (*Bombus*) in the North American Midwest, *Biol. Conserv.* 142, 75–84.
- Hanski I., Saccheri I. (2006) Molecular-Level Variation Affects Population Growth in a Butterfly Metapopulation, *PLoS Biol.* 4, e129.
- Harbo J.R., Harris J.W. (1999) Heritability in honey bees (Hymenoptera: Apidae) of characteristics associated with resistance to *Varroa jacobsoni* (Mesostigmata: Varroidae), *J. Econ. Entomol.* 92, 261–265.
- Hasselmann M., Gempe T., Schiott M., Nunes-Silva C.G., Otte M., Beye M. (2008) Evidence for the evolutionary nascence of a novel sex determination pathway in honeybees, *Nature* 454, 519–522.
- Hebert P.N.D., Ratnasingham S., DeWaard J.R. (2003) Barcoding animal life: cytochrome *c* oxidase subunit I divergences among closely related species, *Proc. R. Soc. Ser. B Biol. Sci.* 270, S96–S99.
- Hedrick P.W. (2000) *Genetics of Populations*, Second edn, Jones and Bartlett Publishers, Sudbury, Massachusetts.
- Hedrick P.W. (2004) Recent developments in conservation genetics, *For. Ecol. Manage.* 197, 3–19.
- Hedrick P.W., Gilpin M.E. (1997) Genetic effective size of a metapopulation, in: Hanski I.A., Gilpin M.E. (Eds.), *Metapopulation biology: ecology, genetics, and evolution*, Academic Press Inc. San Diego, USA, pp. 165–181.
- Hedrick P.W., Parker J.D. (1997) Evolutionary genetics and genetic variation of haplodiploids and X-lined genes, *Annu. Rev. Ecol. Syst.* 28, 55–83.
- Hedrick P.W., Kalinowski S.T. (2000) Inbreeding depression in conservation biology, *Annu. Rev. Ecol. Syst.* 31, 139–162.
- Hedrick P.W., Gadau J., Page R.E.J. (2006) Genetic sex determination and extinction, *Trends Ecol. Evol.* 21, 55–57.
- Heimpel G.E., de Boer J.G. (2008) Sex determination in the Hymenoptera, *Annu. Rev. Entomol.* 53, 209–230.
- Henter H.J. (2003) Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa, *Evolution* 57, 1793–1803.
- Herrmann F., Westphal C., Moritz R.F.A., Steffan-Dewenter I. (2007) Genetic diversity and mass resources promote colony size and forager densities of a social bee (*Bombus pascuorum*) in agricultural landscapes, *Mol. Ecol.* 16, 1167–1178.
- Holehouse K.A., Hammond R.L., Bourke A.F.G. (2003) Non-lethal sampling of DNA from bumble bees for conservation genetics, *Insectes Soc.* 50, 277–285.
- Holloway A.K., Heimpel G.E., Strand M.R., Antolin M.F. (1999) Survival of diploid males in *Bracon* sp. near *hebetor* (Hymenoptera: Braconidae), *Ann. Entomol. Soc. Am.* 92, 110–116.
- Jacquard A. (1975) Inbreeding: One word, several meanings, *Theor. Popul. Biol.* 7, 338–363.
- Keller L.F., Waller D.M. (2002) Inbreeding effects in wild populations, *Trends Ecol. Evol.* 17, 230–241.
- Kerr W.E. (1987) Sex determination in bees. XXI. Number of XO-heteroalleles in a natural population of *Melipona compressipes fasciculata* (Apidae), *Insectes Soc.* 34, 274–279.
- Kerr W.E. (1997) Sex determination in honey bees (Apinae and Meliponinae) and its consequences, *Braz. J. Genet.* 20, 4.
- Kim S., Misra A. (2007) SNP genotyping: Technologies and biomedical applications, *Annu. Rev. Biomed. Eng.* 9, 289–320.
- Klein A.M., Vaissière B.E., Cane J.H., Steffan-Dewenter I., Cunningham S.A., Kremen C., Tscharntke T. (2007) Importance of pollinators in changing landscapes for world crops, *Proc. R. Soc. Lond., Ser. B Biol. Sci.* 274, 303–313.
- Knight M.E., Martin A.P., Bishop S., Osborne J.L., Hale R.J., Sanderson R.A., Goulson D. (2005) An interspecific comparison of foraging range and nest density of four bumblebee (*Bombus*) species, *Mol. Ecol.* 14, 1811–1820.
- Kosior A., Celary W., Olejniczak P., Fijał J., Król W., Solarz W., Plonka P. (2007) The decline of the bumble bees and cuckoo bees (Hymenoptera: Apidae: Bombini) of western and central Europe, *Oryx* 41, 79–88.
- Kraus F.B., Weinhold S., Moritz R.F.A. (2008) Genetic structure of drone congregations of the stingless bee *Scaptotrigona mexicana*, *Insectes Soc.* 55, 22–27.
- Kremen C., Williams N.M., Thorp R.W. (2002) Crop pollination from native bees at risk from agricultural intensification, *Proc. Natl Acad. Sci. USA* 99, 16812–16816.

- Kremen C., Williams N.M., Aizen M.A., Gemmill-Herren B., LeBuhn G., Minckley R., Packer L., Potts S.G., Roulston T.A., Steffan-Dewenter I., Vazquez D.P., Winfree R., Adams L., Crone E.E., Greenleaf S.S., Keitt T.H., Klein A.-M., Regetz J., Ricketts T.H. (2007) Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change, *Ecol. Lett.* 10, 299–314.
- Krieger M.J.B., Ross K.G., Chang C.W., Keller L. (1999) Frequency and origin of triploidy in the fire ant *Solenopsis invicta*, *Heredity* 82, 142–150.
- Kuhlmann M., Else G.R., Dawson A., Quicke D.L.J. (2007) Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae), *Org. Divers. Evol.* 7, 155–165.
- Kukuk P.F., May B. (1990) Diploid males in a primitively eusocial bee, *Lasioglossum (Dialictus) zephyrum*, *Evolution* 44, 1522–1528.
- Lacy R.C. (1993) Vortex: a computer simulation model for population viability analysis, *Wildl. Res.* 20, 45–64.
- Lapidge K.L., Oldroyd B.P., Spivak M. (2002) Seven suggestive quantitative trait loci influence hygienic behavior of honey bees, *Naturwissenschaften* 89, 565–568.
- Le Conte Y., De Vaublanc G., Crauser D., Jeanne F., Rousselle J.C., Becard J.M. (2007) Honey bee colonies that have survived *Varroa destructor*, *Apidologie* 38, 566–572.
- Leberg P.L., Firmin B.D. (2008) Role of inbreeding depression and purging in captive breeding and restoration programmes, *Mol. Ecol.* 2008, 334–343.
- Liebert A.E., Johnson R.N., Switz G.T., Starks P.T. (2004) Triploid females and diploid males: under-reported phenomena in *Polistes* wasps? *Insectes Soc.* 51, 205–211.
- Liebert A.E., Sumana A., Starks P.T. (2005) Diploid males and their triploid offspring in the paper wasp *Polistes dominulus*, *Biol. Lett.* 1, 200–203.
- Lopez-Urbe M.M., Almanza M.T., Ordóñez M. (2007) Diploid male frequencies in Colombian populations of euglossine bees, *Biotropica* 39, 299–314.
- Lozier J.D., Cameron S.A. (2009) Comparative genetic analyses of historical and contemporary collections highlight contrasting demographic histories for the bumble bees *Bombus pensylvanicus* and *B. impatiens* in Illinois, *Mol. Ecol.*, doi:10.1111/j.1365.294X.2009.04160x.
- Luikart G., England P.R. (1999) Statistical analysis of microsatellite DNA data, *Trends Ecol. Evol.* 14, 253–256.
- Luna M.G., Hawkins B.A. (2004) Effects of Inbreeding Versus Outbreeding in *Nasonia vitripennis* (Hymenoptera: Pteromalidae), *Environ. Entomol.* 33, 765–775.
- Lynch M., Conery J., Burger R. (1995) Mutation accumulation and the extinction of small populations, *Am. Nat.* 146, 489–518.
- Lynch M., Walsh B. (1998) *Genetics and Analysis of Quantitative Traits*, Sinauer Associates Inc., Sunderland, MA, USA.
- Margulies M., Egholm M., Altman W.E., Attiya S., Bader J.S., Bemben L.A., Berka J., Braverman M.S., Chen Y.-J., Chen Z., Dewell S.B., Du L., Fierro J.M., Gomes X.V., Godwin B.C., He W., Helgesen S., Ho C.H., Irzyk G.P., Jando S.C., Alenquer M.L.I., Jarvie T.P., Jirage K.B., Kim J.-B., Knight J.R., Lanza J.R., Leamon J.H., Lefkowitz S.M., Lei M., Li J., Lohman K.L., Lu H., Makhijani V.B., McDade K.E., McKenna M.P., Myers E.W., Nickerson E., Nobile J.R., Plant R., Puc B.P., Ronan M.T., Roth G.T., Sarkis G.J., Simons J.F., Simpson J.W., Srinivasan M., Tartaro K.R., Tomasz A., Vogt K.A., Volkmer G.A., Wang S.H., Wang Y., Weiner M.P., Yu P., Begley R.F., Rothberg J.M. (2005) Genome sequencing in microfabricated high-density picolitre reactors, *Nature* 437, 376–380.
- McCorquodale D.B., Owen R.E. (1994) Laying sequence, diploid males and nest usurpation in the leafcutter bee, *Megachile rotundata* (Hymenoptera: Megachilidae), *J. Insect. Behav.* 7, 731–738.
- Michener C.D. (2000) *The bees of the world*, The Johns Hopkins University Press, Baltimore, Maryland.
- Michener C.D., Rettenmeyer C.W. (1956) The ethology of *Andrena erythronii* with comparative data on other species, *Univ. Kans. Sci. Bull.* 37, 645–684.
- Minckley R.L., Wcislo W.T., Yanega D., Buchmann S.L. (1994) Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability, *Ecology* 75, 1406–1419.
- Mitchell-Olds T., Willis J.H., Goldstein D.B. (2007) Which evolutionary processes influence natural genetic variation for phenotypic traits? *Nat. Rev. Genet.* 8, 845–856.
- Morin P.A., Luikart G., Wayne R.K. (2004) SNPs in ecology, evolution and conservation, *Trends Ecol. Evol.* 19, 208–216.
- Moritz C., Cicero C. (2004) DNA barcoding: promise and pitfalls, *PLoS Biol.* 2, e279.
- Müller A., Diener S., Schnyder S., Stutz K., Sedivy C., Dorm S. (2006) Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee–flower relationships, *Biol. Conserv.* 130, 604–615.
- Murray T.E., Fitzpatrick U., Brown M.J.F., Paxton R.J. (2008) Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs, *Conserv. Genet.* 9, 653–666.

- Murray T.E., Kuhlmann M., Potts S.G. (2009) Conservation ecology of bees: populations, species and communities, *Apidologie* 40, 211–236.
- Navajas M., Migeon A., Alaux C., Martin-Magniette M.L., Robinson G.E., Evans J.D., Cros-Arteil S., Crauser D., Le Conte Y. (2008) Differential gene expression of the honey bee *Apis mellifera* associated with *Varroa destructor* infection, *BMC Genomics* 9, 301.
- Neumann N.F., Galvez F. (2002) DNA microarrays and toxicogenomics: applications for ecotoxicology? *Biotechnol. Adv.* 20, 391–419.
- Nevins J.R., Potti A. (2007) Mining gene expression profiles: expression signatures as cancer phenotypes, *Nat. Rev. Genet.* 8, 601–609.
- Nielsen R. (2005) Molecular signatures of natural selection, *Annu. Rev. Genet.* 39, 197–218.
- Nielsen R., Hellmann I., Hubisz M., Bustamante C., Clark A.G. (2007) Recent and ongoing selection in the human genome, *Nat. Rev. Genet.* 8, 857–868.
- NRC (2007) Status of pollinators in North America, National Academies Press, Washington, DC.
- Olesen J.M., Bascompte J., Dupont Y.L., Jordano P. (2007) The modularity of pollination networks, *Proc. Natl Acad. Sci. USA* 104, 19891–19896.
- Otterstatter M.C., Thomson J.D. (2008) Does Pathogen Spillover from Commercially Reared Bumble Bees Threaten Wild Pollinators? *PLoS ONE* 3, e2771.
- Owen R.E. (1985) The opportunity for polymorphism and genic variation in social hymenoptera with worker-produced males, *Heredity* 54, 25–36.
- Owen R.E., Packer L. (1994) Estimation of the proportion of diploid males in populations of Hymenoptera, *Heredity* 72, 219–227.
- Packer L., Owen R.E. (1990) Allozyme variation, linkage disequilibrium and diploid male production in a primitively social bee *Augochlorella striata* (Hymenoptera: Halictidae), *Heredity* 65, 241–248.
- Packer L., Owen R. (2001) Population genetic aspects of pollinator decline, *Conserv. Ecol.* 5, 4.
- Packer L., Zayed A., Grixti J.C., Ruz L., Owen R.E., Vivallo F., Toro H. (2005) Conservation genetics of potentially endangered mutualisms: reduced levels of genetic variation in specialist versus generalist bees, *Conserv. Biol.* 19, 195–202.
- Page R.E. (1980) The evolution of multiple mating behavior by honey bee queens (*Apis mellifera*), *Genetics* 96, 263–273.
- Palmer K.A., Oldroyd B.P., Quezada-Euán J.J.G., Paxton R.J., May-Itza W.D.J. (2002) Paternity frequency and maternity of males in some stingless bee species, *Mol. Ecol.* 11, 2107–2113.
- Pamilo P., Crozier R.H. (1997) Population biology of social insect conservation, *Mem. Mus. Vict.* 56, 411–419.
- Pauw A. (2007) Collapse of a pollination web in small conservation areas, *Ecology* 88, 1759–1769.
- Paxton R.J. (2005) Male mating behaviour and mating systems of bees: an overview, *Apidologie* 36, 145–156.
- Paxton R.J., Thorén P.A., Gyllenstrand N., Tengö J. (2000) Microsatellite DNA analysis reveals low diploid male production in a communal bee with inbreeding, *Biol. J. Linn. Soc. Lond.* 69, 483–502.
- Paxton R.J., Bego L.R., Shah M.M., Mateus S. (2003) Low mating frequency of queens in the stingless bee *Scaptotrigona postica* and worker maternity of males, *Behav. Ecol. Sociobiol.* 53, 174–181.
- Pfrender M.E., Spitze K., Hicks J., Morgan K., Latta L., Lynch M. (2000) Lack of concordance between genetic diversity estimates at the molecular and quantitative-trait levels, *Conserv. Genet.* 1, 263–269.
- Plowright R.C., Pallett M.J. (1979) Worker-male conflict and inbreeding in bumble bees (Hymenoptera: Apidae), *Can. Entomol.* 111, 289–294.
- Quezada-Euán J.J.G., Paxton R.J., Palmer K.A., Itza W.D.M., Tay W.T., Oldroyd B.P. (2007) Morphological and molecular characters reveal differentiation in a Neotropical social bee, *Melipona beecheii* (Apidae: Meliponini), *Apidologie* 38, 247–258.
- Ralls K., Ballou J. (1983) Extinction: lessons from zoos, in: Schonewald-Cox C.M., Chambers S.M., MacBryde B., Thomas L. (Eds.), *Genetics and Conservation: A Reference for Managing Wild Animal and Plant Populations*, Benjamin/Cummings Menlo Park, CA.
- Ralls K., Ballou J.D., Templeton A. (1988) Estimates of lethal equivalents and the cost of inbreeding in mammals, *Conserv. Biol.* 2, 185–193.
- Reed D.H., Frankham R. (2001) How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis, *Evolution* 55, 1095–1103.
- Reed D.H., Frankham R. (2003) Correlation between fitness and genetic diversity, *Conserv. Biol.* 17, 230–237.
- Ross K.G., Fletcher D.J.C. (1986) Diploid male production - a significant colony mortality factor in the fire ant *Solenopsis invicta* (Hymenoptera: Formicidae), *Behav. Ecol. Sociobiol.* 19, 283–291.
- Roubik D.W. (2001) Ups and Downs in Pollinator Populations: When is there a Decline? *Conserv. Ecol.* 5, 2. [online] <http://www.consecol.org/vol5/iss1/art2> (accessed on 2 February 2009).

- Roubik D.W., Weigt L.A., Bonilla M.A. (1996) Population genetics, diploid males, and limits to social evolution of euglossine bees, *Evolution* 50, 931–935.
- Sabeti P.C., Schaffner S.F., Fry B., Lohmueller J., Varilly P., Shamovsky O., Palma A., Mikkelsen T.S., Altshuler D., Lander E.S. (2006) Positive natural selection in the human lineage, *Science* 312, 1614–1620.
- Saccheri I., Kuussaari M., Kankare M., Vikman P., Fortelius W., Hanski I. (1998) Inbreeding and extinction in a butterfly metapopulation, *Nature* 392, 491–494.
- Schena M., Shalon D., Davis R.W., Brown P.O. (1995) Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray, *Science* 270, 467–470.
- Schmid-Hempel P., Schmid-Hempel R., Brunner P.C., Seeman O.D., Allen G.R. (2007) Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck, *Heredity* 99, 414–422.
- Schremph A., Aron S., Heinze J. (2006) Sex determination and inbreeding depression in an ant with regular sib-mating, *Heredity* 97, 75–80.
- Shendure J., Mitra R.D., Varma C., Church G.M. (2004) Advanced sequencing technologies: methods and goals, *Nat. Rev. Genet.* 5, 335–344.
- Simmonds F.J. (1947) Improvement of the sex ratio of a parasite by selection, *Can. Entomol.* 79, 41–44.
- Spielman D., Brook B.W., Frankham R. (2004) Most species are not driven to extinction before genetic factors impact them, *Proc. Natl Acad. Sci. USA* 101, 15261–15264.
- Stouthamer R., Luck R.F., Werren J.H. (1992) Genetics of sex determination and the improvement of biological control using parasitoids, *Environ. Entomol.* 21, 427–435.
- Stow A., Silberbauer L., Beattie A.J., Briscoe D.A. (2007) Fine-scale genetic structure and fire-created habitat patchiness in the Australian allopapine bee, *Exoneura nigrescens* (Hymenoptera: Apidae), *J. Hered.* 98, 60–66.
- Strange J.P., Knoblett J., Griswold T. (2009) DNA amplification from pin-mounted bumble bees (*Bombus*) in a museum collection: effects of fragment size and specimen age on successful PCR, *Apidologie*, DOI: 10.1051/apido/2008070.
- Strassmann J. (2001) The rarity of multiple mating by females of social Hymenoptera, *Insectes Soc.* 48, 1–13.
- Strausberg R.L., Levy S., Rogers Y.-H. (2008) Emerging DNA sequencing technologies for human genomic medicine, *Drug Discov. Today* 13, 569–577.
- Takahashi N.C., Peruquetti R.C., Del Lama M.A., de Oliveira Campos L.A. (2001) A reanalysis of diploid male frequencies in euglossine bees (Hymenoptera: Apidae), *Evolution* 55, 1897–1899.
- Takahashi J., Ayabe T., Mitsuhashi M., Shimizu I., Ono M. (2008) Diploid male production in a rare and locally distributed bumblebee, *Bombus florilegus* (Hymenoptera, Apidae), *Insectes Soc.* 55, 43–50.
- Tavares M.G., Dias L.A.D., Borges A.A., Lopes D.M., Busse A.H.P., Costa R.G., Salomao T.M.F., Campos L.A.D. (2007) Genetic divergence between populations of the stingless bee uruqu amarela (*Melipona rufiventris* group, Hymenoptera, Meliponini): Is there a new *Melipona* species in the Brazilian state of Minas Gerais? *Genet. Mol. Biol.* 30, 667–675.
- Templeton A.R., Read B. (1994) Inbreeding: one word, several meanings, much confusion, in: Loeschcke V., Tomiuk J., Jain S.K. (Eds.), *Conservation Genetics*, Birkhäuser-Verlag Basel, Switzerland, pp. 91–106.
- The Honeybee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*, *Nature* 443, 931–949.
- Twyman R.M. (2004) SNP discovery and typing technologies for pharmacogenomics, *Curr. Top. Med. Chem.* 4, 1423–1431.
- Vamosi J.C., Knight T.M., Steets J.A., Mazer S.J., Burd M., Ashman T.-L. (2006) Pollination decays in biodiversity hotspots, *Proc. Natl Acad. Sci. USA* 103, 956–961.
- van't Veer L.J., Bernards R. (2008) Enabling personalized cancer medicine through analysis of gene-expression patterns, *Nature* 452, 564–570.
- van Wilgenburg E., Driessen G., Beukeboom L. (2006) Single locus complementary sex determination in Hymenoptera: an “unintelligent” design? *Front. Zool.* 3, 1–15.
- Vilas C., San Miguel E., Amaro R., Garcia C. (2006) Relative contribution of inbreeding depression and eroded adaptive diversity to extinction risk in small populations of shore *Campion*, *Conserv. Biol.* 20, 229–238.
- Wadlow R., Ramaswamy S. (2005) DNA microarrays in clinical cancer research, *Curr. Mol. Med.* 5, 111–120.
- Wandeler P., Hoek P.E.A., Keller L.F. (2007) Back to the future: museum specimens in population genetics, *Trends Ecol. Evol.* 22, 634–642.
- Wang J. (2005) Estimation of effective population sizes from data on genetic markers, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 360, 1395–1409.
- Werren J.H. (1993) The evolution of inbreeding in haplodiploid organisms, in: Thornhill N.W. (Ed.), *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*, The University of Chicago Press Chicago, pp. 42–59.

- Westemeier R.L., Brawn J.D., Simpson S.A., Esker T.L., Jansen R.W., Walk J.W., Kershner E.L., Bouzat J.L., Paige K.N. (1998) Tracking the long-term decline and recovery of an isolated population, *Science* 282, 1695–1698.
- Whitfield C.W., Band M.R., Bonaldo M.F., Kumar C.G., Liu L., Pardinas J.R., Robertson H.M., Soares M.B., Robinson G.E. (2002) Annotated Expressed Sequence Tags and cDNA Microarrays for Studies of Brain and Behavior in the Honey Bee, *Genome Res.* 12, 555–566.
- Whitfield C.W., Behura S.K., Berlocher S.H., Clark A.G., Johnston J.S., Sheppard W.S., Smith D.R., Suarez A.V., Weaver D., Tsutsui N.D. (2006) Thrice Out of Africa: Ancient and Recent Expansions of the Honey Bee, *Apis mellifera*, *Science* 314, 642–645.
- Whitlock M.C., Barton N.H. (1997) The effective size of a subdivided population, *Genetics* 146, 427–441.
- Widmer A., Schmid-Hempel P. (1999) The population genetic structure of a large temperate pollinator species, *Bombus pascuorum* (Scopoli) (Hymenoptera: Apidae), *Mol. Ecol.* 8, 387–398.
- Wilfert L., Gadau J., Baer B., Schmid-Hempel P. (2007a) Natural variation in the genetic architecture of a host-parasite interaction in the bumblebee *Bombus terrestris*, *Mol. Ecol.* 16, 1327–1339.
- Wilfert L., Gadau J., Schmid-Hempel P. (2007b) The genetic architecture of immune defense and reproduction in male *Bombus terrestris* bumblebees, *Evolution* 61, 804–815.
- Williams N., Minckley R., Silveira F. (2001) Variation in native bee faunas and its implications for detecting community changes, *Conserv. Ecol.* 5, 7.
- Woyke J. (1979) Sex determination in *Apis cerana indica*, *J. Apic. Res.* 18, 122–127.
- Wu Z., Hopper K.R., Ode P.J., Fuester R.W., Chen J., Heimpel G.E. (2003) Complementary Sex Determination In Hymenopteran Parasitoids And Its Implications For Biological Control., *Entomol. Sin.* 10, 81–93.
- Yokoyama S., Nei M. (1979) Population dynamics of sex-determining alleles in honey bees and self-incompatibility alleles in plants, *Genetics* 91, 609–626.
- Zayed A. (2004) Effective population size in Hymenoptera with complementary sex determination, *Heredity* 93, 627–630.
- Zayed A., Packer L. (2001) High levels of diploid male production in a primitively eusocial bee (Hymenoptera: Halictidae), *Heredity* 87, 631–636.
- Zayed A., Packer L. (2005) Complementary sex determination substantially increases extinction proneness of haplodiploid populations, *Proc. Natl Acad. Sci. USA* 102, 10742–10746.
- Zayed A., Packer L. (2007) The population genetics of a solitary oligolectic sweat bee, *Lasioglossum (Sphecodogastra) oenotherae* (Hymenoptera: Halictidae), *Heredity* 99, 397–405.
- Zayed A., Whitfield C.W. (2008) A genome-wide signature of positive selection in ancient and recent invasive expansions of the honey bee *Apis mellifera*, *Proc. Natl Acad. Sci. USA* 105, 3421–3426.
- Zayed A., Roubik D.W., Packer L. (2004) Use of diploid male frequency data as an indicator of pollinator decline, *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 271, S9–S12.
- Zayed A., Packer L., Grixti J.C., Ruz L., Toro H., Owen R.E. (2005) Increased genetic differentiation in a specialist versus a generalist bee: implications for conservation, *Conserv. Genet.* 6, 1017–1026.
- Zayed A., Constantin S.A., Packer L. (2007) Successful biological invasion despite a severe genetic load, *PLoS ONE* 2, e868.