



Hybrid origins of Australian honeybees (*Apis mellifera*)

Nadine C. Chapman, Brock A. Harpur, Julianne Lim, Thomas E. Rinderer,
Michael H. Allsopp, Amro Zayed, Benjamin P. Oldroyd

► To cite this version:

Nadine C. Chapman, Brock A. Harpur, Julianne Lim, Thomas E. Rinderer, Michael H. Allsopp, et al.. Hybrid origins of Australian honeybees (*Apis mellifera*). *Apidologie*, 2016, 47 (1), pp.26-34. 10.1007/s13592-015-0371-0 . hal-01332462

HAL Id: hal-01332462

<https://hal.science/hal-01332462>

Submitted on 15 Jun 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Hybrid origins of Australian honeybees (*Apis mellifera*)

Nadine C. CHAPMAN¹, Brock A. HARPUR², Julianne LIM¹, Thomas E. RINDERER³,
Michael H. ALLSOPP⁴, Amro ZAYED², Benjamin P. OLDROYD¹

¹Behaviour and Genetics of Social Insects Laboratory, School of Biological Sciences A12, University of Sydney, Sydney, NSW 2006, Australia

²Department of Biology, York University, 4700 Keele Street, Toronto, Ontario, Canada M3J 1P3

³Honey-Bee Breeding, Genetics and Physiology Research Laboratory, USDA-ARS, 1157 Ben Hur Road, Baton Rouge, LA 70820, USA

⁴ARC-Plant Protection Research Institute, Stellenbosch 7599, South Africa

Received 12 October 2014 – Revised 12 April 2015 – Accepted 13 May 2015

Abstract – With increased globalisation and homogenisation, the maintenance of genetic integrity in local populations of agriculturally important species is of increasing concern. The western honeybee (*Apis mellifera*) provides an interesting perspective as it is both managed and wild, with a large native range and much larger introduced range. We employed a newly created 95 single nucleotide polymorphism (SNP) test to characterise the genetic ancestry of the Australian commercial and feral honeybee populations. We found that most individuals were hybrids of mainly Western and Eastern European ancestry. Introductions of bees from North Africa are known from the historical record, and we show here the presence of alleles of African ancestry in some Australian bees, at levels comparable to those seen in the commercial populations of European-derived bees in North America.

Australia / beekeeping / single nucleotide polymorphisms / honeybee subspecies / hybrid

1. INTRODUCTION

Maintaining genetic diversity in domesticated animals is of increasing concern (Hall 2004; Ajmone-Marsan & GLOBALDIV Consortium 2010; Groeneveld et al. 2010; Lenstra et al. 2012). In general, domestication leads to a loss of genetic diversity, although this is not evident in the Western honeybee *Apis mellifera* (Whitfield et al. 2006; Harpur et al. 2012, 2014; Wallberg et al. 2014). Nevertheless there are legitimate concerns over the loss of genetic diversity and increasing homogenisation in *A. mellifera*, both

between and within populations (De la Rua et al. 2009; Dietemann et al. 2009; Meixner et al. 2010; De la Rua et al. 2013; Harpur et al. 2013), and the maintenance of genetic diversity within colonies, which increases honey production, nest homeostasis and disease resistance (Palmer & Oldroyd 2003; Tarpy 2003; Tarpy & Seeley 2006; Mattila & Seeley 2007; Oldroyd & Fewell 2007; Seeley & Tarpy 2007; Tarpy et al. 2013).

Though its native range was originally confined to Africa, Europe and the Middle East (Seeley 1985; Winston 1987; Ruttner 1988), the distribution of *A. mellifera* now extends over most of the world. The honeybee was spread by humans for its value in honey and wax production and crop pollination. It has been estimated that 84 % of world crops depend on insect pollinators, including honeybees (Klein et al. 2007). In Australia, 35 industries and 65 % of agricultural production are wholly or partially dependent on honeybee pollination (Keogh et al. 2010).

Electronic supplementary material The online version of this article (doi:10.1007/s13592-015-0371-0) contains supplementary material, which is available to authorized users.

Corresponding author: N. Chapman,
nadine.chapman@sydney.edu.au
Handling Editor: Marina Meixner

There is considerable controversy over the number of evolutionary lineages and subspecies within *A. mellifera*, and which subspecies belong to which lineage (Ruttner 1988). Four major lineages (clades) are generally recognised—African (A), Eastern European (C), Western European (M) and Middle Eastern (O) (Ruttner 1988; Garnery et al. 1992; Franck et al. 2000; Whitfield et al. 2006; Harpur et al. 2014; Wallberg et al. 2014) although up to two additional lineages have been suggested (Franck et al. 2001; Alburaki et al. 2011; Alburaki et al. 2013). The commonly used subspecies by commercial beekeepers are *A. m. ligustica* and *A. m. carnica* from the Eastern European lineage.

A number of subspecies have been introduced to Australia. The earliest imports were from Western Europe—*A. m. mellifera* (and perhaps *A. m. iberiensis* (*iberica*)) Chapman et al. 2008). Descendants of these bees quickly spread throughout the country (Coleman 1956; Ruttner 1976; Weatherhead 1986; Oldroyd et al. 1995). Several decades later, *A. m. ligustica* (early 1860s) and *A. m. carnica* (from 1890s) were introduced from Eastern Europe as well as *A. m. caucasica* from the O lineage (from 1880s) (Hopkins 1886; Eckert 1958; Weatherhead 1986; Woodward 1993; Gulliford 2005; Rhodes 2011), along with *A. m. intermissa* and *A. m. lamarckii* from Africa in the late nineteenth century (Weatherhead 1986; Gulliford 2005; Rhodes 2011), *A. m. cypria* from Cyprus between 1896 and 1952 (Goodacre 1935; Gulliford 2005) and *A. m. syriaca* from Syria in the late nineteenth century (Gulliford 2005; Rhodes 2011) and perhaps others not known from the historical record. Today, the favoured subspecies in Australia are ‘Italian’ or ‘Ligurian’ (*A. m. ligustica*), ‘Carniolan’ (*A. m. carnica*) and ‘Caucasian’ (*A. m. caucasica*). An online search for ‘Australian bee breeders’ yields 13 breeders, ten who propagate *A. m. ligustica*, four *A. m. caucasica* and one *A. m. carnica*, while two did not specify a subspecies.

Genetic studies of Australian bees have identified signatures of the European lineages only (Oldroyd et al. 1993; 1995; Koulouanos & Crozier 1996, 1997; Chapman et al. 2008; Oxley & Oldroyd 2009). The bees from Kangaroo Island,

20 km off the South Australian coast, are still reputed to be the most ‘pure’ *A. m. ligustica* strain in the world, despite carrying mitochondria of *A. m. mellifera* origin (Oldroyd et al. 1993; Koulouanos & Crozier 1996, 1997) and allozymes at frequencies associated with *A. m. siciliana* (Oldroyd et al. 1993). Bees from the Tasmanian highlands have previously been characterised as being of *A. m. mellifera* origin (Oldroyd et al. 1995; Koulouanos & Crozier 1996, 1997), while other, warmer, Tasmania regions have a mix of *A. m. ligustica* and some *A. m. mellifera* (Oldroyd et al. 1995; Koulouanos & Crozier 1997). Mitotypes consistent with *A. m. ligustica* (Western Australia [WA], New South Wales [NSW], Victoria [VIC], Tasmania [TAS]), *A. m. caucasica* (NSW) and *A. m. mellifera* (WA, Kangaroo Island [KI], TAS, VIC, NSW) have been found in Australia (Koulouanos & Crozier 1997). Mitochondrial sequences associated with *A. m. ligustica* (commercial population) and *A. m. iberiensis* (commercial and feral population) have been identified in WA (Chapman et al. 2008) while those of *A. m. ligustica*, *A. m. carnica* and *A. m. caucasica*, and possibly *A. m. macedonica*, have been identified in commercial colonies from the other states (Oxley & Oldroyd 2009).

In this study, we examine both feral and commercial Australian honeybees with 95 single nucleotide polymorphism (SNP) markers newly created to differentiate between the African, Western European and Eastern European lineages (Chapman et al. 2015) in order to obtain a genetic profile of Australian honeybees.

2. METHODS

We obtained samples from 102 colonies from the commercial population (Figure 1; [Supplementary Information](#)) and 104 feral (unmanaged) samples from both colonies and drone congregation areas (Figure 2; [Supplementary Information](#)). DNA was extracted from one individual per colony with phenol-chloroform isoamyl alcohol (Sambrook et al. 1989) and typed at 95 SNPs (Chapman et al. 2015) using the Sequenom MassARRAY MALDI-TOF system (Sequenom, CA, USA). The 95 SNPs were chosen on the basis of high pairwise F_{ST} values between the African, Western

European and Eastern European lineages (Harpur et al. 2014) or because they were hypothesised to be under selection (Whitfield et al. 2006), had minor allele frequencies greater than 5 % and more than two thirds of genotypes successfully typed (Chapman et al. 2015). SNPs were unlinked; on average, SNPs were separated by 1,734,863 base pairs and the closest SNPs were 45,945 base pairs apart (Chapman et al. 2015). Haploid data were coded as missing data at one allele for each SNP marker (Pritchard et al. 2010). Ancestry was assigned to individuals based on reference populations from the Western European (M; *A. m. mellifera* and *A. m. iberiensis*; $n=13$), Eastern European (C; *A. m. carnica* and *A. m. ligustica*; $n=86$) and African (A; *A. m. scutellata*; $n=128$) lineages (Chapman et al. 2015) using STRUCTURE (v2.3.4 Pritchard et al. 2000) with three ancestral lineages assumed (but not given a priori) and a burn-in phase of 50,000 iterations followed by 100,000 iterations with admixture assumed and uncorrelated allele frequencies.

3. RESULTS

The commercial and feral populations of Australia carry a similar (low) proportion of alleles from the African lineage (3.83 versus 3.97 %). The commercial population carries more Eastern European alleles (69.36 versus 57.39 %) and fewer Western European alleles (26.81 versus 38.64 %) than the feral population, although the difference in ancestry proportions from the three lineages between the two populations was not statistically significant ($\chi^2_2=3.271$, $P=0.195$; Figure 3).

The bees from the highlands in the centre of Tasmania have a high proportion of Western European alleles (commercial, 86.3–92.0 %, Figure 1; feral, 81.1–90.0 %, Figure 2). In contrast, bees collected from elsewhere in Tasmania (commercial, 11.7–30.9 %; Figure 1) and Australia (commercial, 1.0–53.1 %; feral, 10.9–77.5 %) have a much lower frequency of alleles of Western European origin. A number of feral samples (seven NSW, three VIC, one SA and seven WA) had a high proportion (>50 %) of Western European alleles (Figure 2), while only two (VIC) commercial individuals had more than 50 % alleles of Western European ancestry (Figure 1). Few individuals could be considered ‘pure’ in

either population. In the commercial population, only three individuals had greater than 90 % Eastern European alleles (two NSW and one SA) and another three had greater than 90 % Western European alleles (all from the Tasmanian highlands; Figure 1), while in the feral population, one individual carried 90 % Western European alleles (from the Tasmania highlands; Figure 2).

Most individuals had few alleles that are present at high frequency in the African lineage reference population *A. m. scutellata* (Figures 1 and 2). In the commercial population, 80.77 % of individuals carried fewer than 5 % African alleles, while in the feral population, it was 75.49 % (Figure 4). Only 6.73 % of commercial samples carried greater than 15 % African alleles, while in the feral population, it was 4.90 % (Figure 4).

4. DISCUSSION

Using a SNP panel designed to differentiate between the African, Eastern European and Western European lineages (Chapman et al. 2015), we found that the commercial and feral honeybee populations of Australia are hybrids of mainly Eastern European and Western European ancestry. While the feral population generally carries more Western European M alleles than the commercial population (38.64 versus 26.81 %), the difference is not statistically significant. This is expected as the first reported introduction of honeybees into Australia were of *A. m. mellifera* from Western Europe, which were allowed to go wild and spread quickly across the country (Ruttner 1976; Weatherhead 1986; Oldroyd et al. 1995), and thus, feral populations have repeatedly been found to be of predominantly Western European origin (Oldroyd et al. 1995; Koulianos & Crozier 1997; Chapman et al. 2008). It is noteworthy that these alleles have persisted in the feral population despite continuous input of Eastern European lineage alleles from the commercial population for more than 100 years. This may suggest that the feral population is largely independent of the commercial population—as has previously been noted in Western Australia (Chapman et al. 2008). Alternatively, selection may be acting on the unmanaged feral population, such that genes with a selective advantage derived

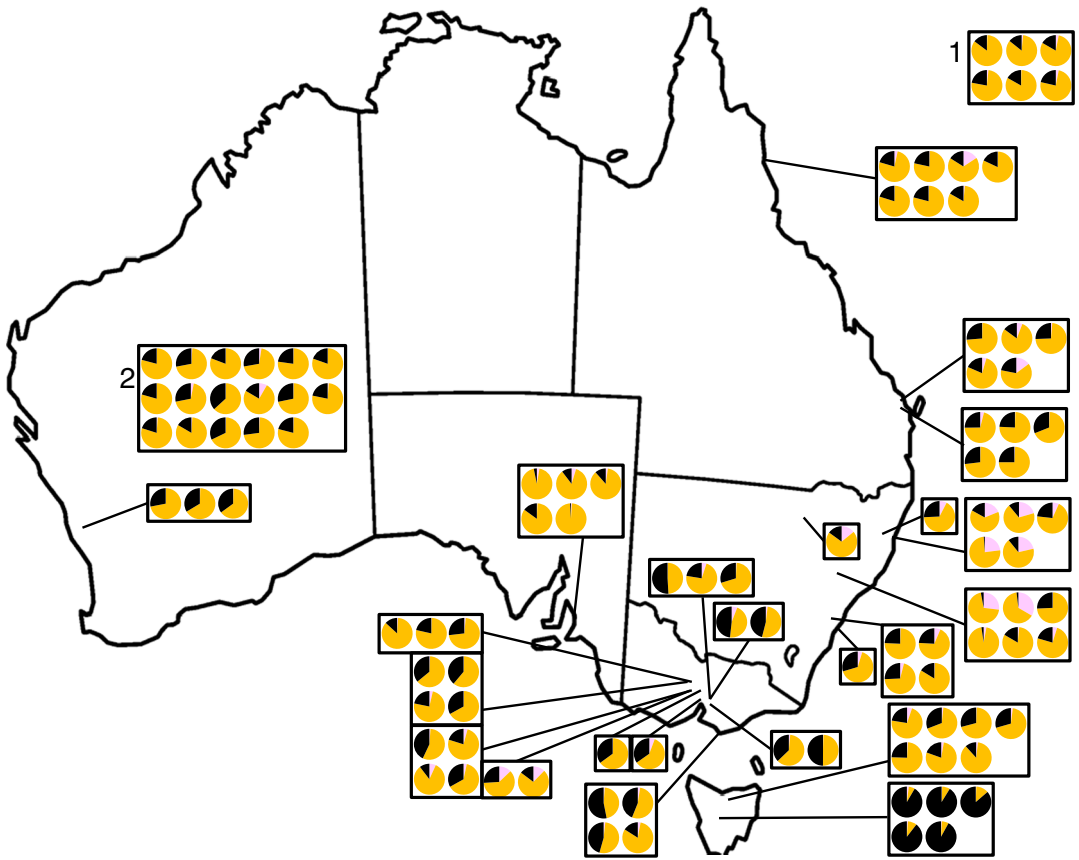


Figure 1. Geographical distribution of sampled individuals from the commercial Australian population with the proportion of ancestry from Eastern European (C; yellow), Western European (M; black) and African (A; pink) lineages. 1 Samples from Norfolk Island, 1412 km east of mainland Australia. 2 Samples from the Western Australian Better Bees breeding programme (Chapman et al. 2008) for which no location is given.

from the Western European lineage are maintained in the feral population, making them more adapted to the local environment (Harpur et al. 2014). Such a pattern has been found in the Africanized population from Brazil and USA, where some alleles from the Western European lineage are retained but those from the Eastern European lineage tend to be lost (Schneider et al. 2004; Harrison et al. 2006; Whitfield et al. 2006; Harpur et al. 2012; Wallberg et al. 2014). These Western European alleles may not be retained in the commercial Australian population as beekeepers select for productivity and other attributes that may be at a selective disadvantage in unmanaged populations. Selection may also be acting to retain the African alleles given their persistence

and the limited historical introduction of bees from North Africa.

For the first time, we found a genetic signature associated with reference samples from Africa in Australia. The historical record suggests that there were a small number of introductions of bees from North Africa to Australia in the late nineteenth and early twentieth centuries (Weatherhead 1986; Gulliford 2005; Rhodes 2011). The African reference samples are *A. m. scutellata*, which has not been imported to Australia, and the identification of alleles associated with Africa does not imply that *A. m. scutellata* was ever imported. There were also some limited introductions from the Middle East (Goodacre 1935; Gulliford 2005), and more extensively of *A. m. caucasia* which

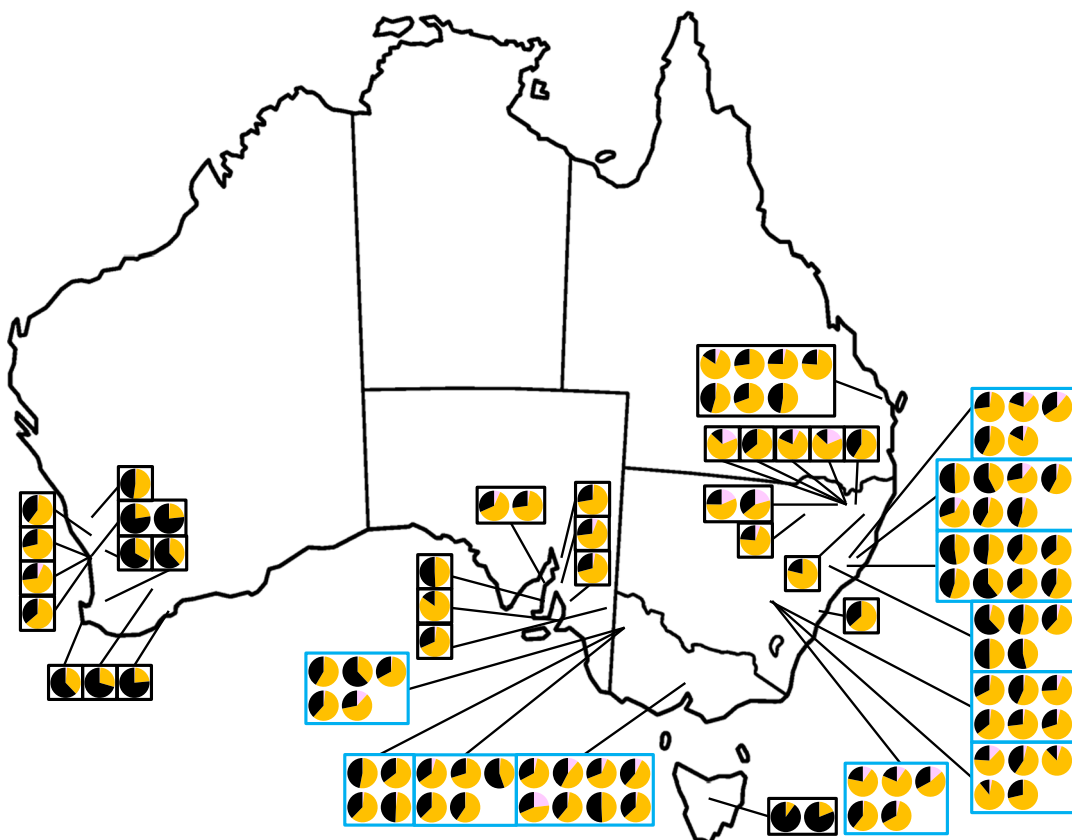


Figure 2. Geographical distribution of sampled individuals from the feral Australian population depicting the proportion of ancestry from Eastern Europe (C; yellow), Western Europe (M; black) and Africa (A; pink). Sampling locations outlined in blue were sampled by collecting drones at drone congregation areas.

has been identified as both Eastern European and Middle Eastern. The Middle Eastern lineage (O) derived from the Eastern European (C) lineage, but has had more recent (in evolutionary time) hybridization with the African (A) lineage (Wallberg et al. 2014). Thus, it is likely that some of the African alleles detected were introduced through importations from the Middle East. A similar proportion of African ancestry has also been shown in commercial populations of the USA, Canada and Europe (Schiff et al. 1994; Whitfield et al. 2006; Wallberg et al. 2014; Chapman et al. 2015). It is clear that these bees are not ‘Africanized’ as populations of Africanized bees from Brazil carry 80.33 % African alleles on average when genotyped with this SNP panel, while those from America carry 62.42 % on average (Chapman et al. 2015).

Our data and the historical record of imports of honeybees into Australia indicate that like the North American population, the Australian population is likely to be highly hybridised and genetically diverse (Harpur et al. 2012, 2013). While Australian bee breeders tend to advertise queens of a particular subspecies, most often this is not true because commercial queens mate with a proportion of feral drones or any other strains that are in the area. Genetic diversity provides opportunities for selection of desirable traits and may benefit individual colonies via direct heterosis (Cale & Gowen 1956; Oldroyd et al. 1985; Oldroyd & Moran 1987; Oldroyd et al. 1987) and social heterosis wherein mixtures of genotypes within colonies can improve the task allocation system and colony performance (Oldroyd et al. 1992; Jones et al. 2004; Chapman et al. 2007; Mattila

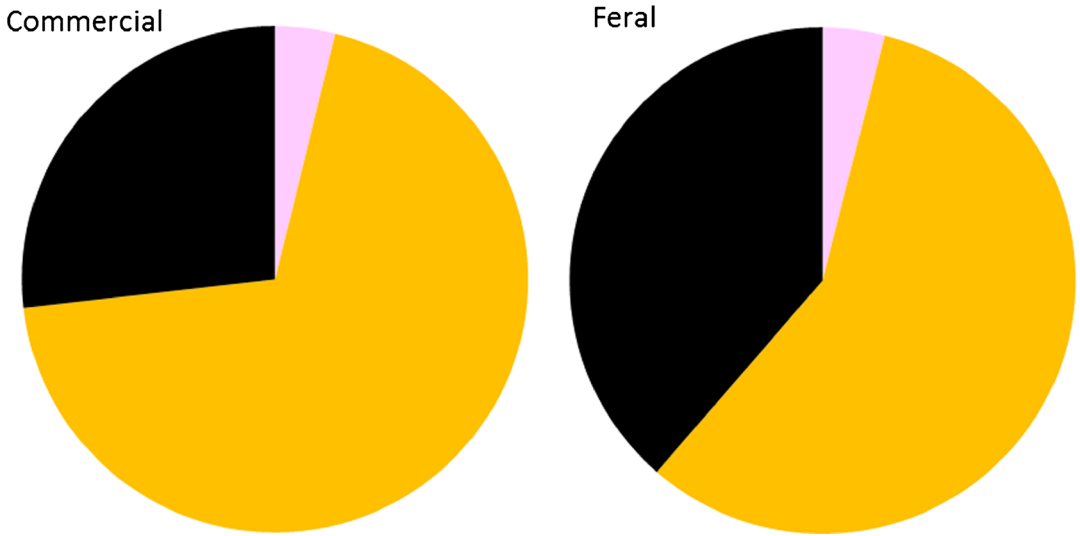


Figure 3. Proportion of ancestry from Eastern European (C; yellow), Western European (M; black) and African (A; pink) lineages in the commercial and feral Australian populations.

& Seeley 2007; Oldroyd & Fewell 2007) and disease resistance (Palmer & Oldroyd 2003; Tarpy 2003; Tarpy & Seeley 2006; Seeley & Tarpy 2007). Thus, the hybrid origin of bees introduced to Australia is likely to promote the health and productivity of Australian honeybees; if a limited number of colonies from a single site had been introduced, then we are likely to have experienced problems with inbreeding. Concerns over inbreeding have been addressed in previous studies (Chapman et al. 2008; Oxley & Oldroyd 2009).

Within the native range of the honeybee, there are strong arguments for conservation of local ecotypes as sources of genetic diversity, including

local adaptations to environmental conditions and pathogens (Strange et al. 2007; De la Rua et al. 2009; Meixner et al. 2010; Oleska et al. 2011; Chavez-Galarza et al. 2013; De la Rua et al. 2013; Harpur et al. 2013; Pinto et al. 2014). The cooperation of local beekeepers in keeping only the desired strain is central to the success of such conservation programmes (Jensen et al. 2005; Jensen & Pedersen 2005). The SNP panel used in this study provides a new tool to measure the success of such conservation programmes and to identify the ancestry of introduced populations.

ACKNOWLEDGMENTS

This project was supported by grants from Rural Industries Research and Development Corporation PRJ-007774 Australia (BPO), a Natural Sciences and Engineering Research Council Discover grant (AZ) and an Ontario Ministry of Research and Innovations Early Researcher Award (AZ). We thank the Australian Department of Agriculture, Fisheries and Forestry, Plant Health Australia, Biosecurity Australia and many beekeepers for contributing samples. We thank the team at Australian Cancer Research Fund at the Garvan Institute for use of their facilities.

Author contributions BPO, AZ and NCC designed the experiment. NCC and JL extracted DNA. NCC performed genotyping and data analysis. AZ and BAH provided

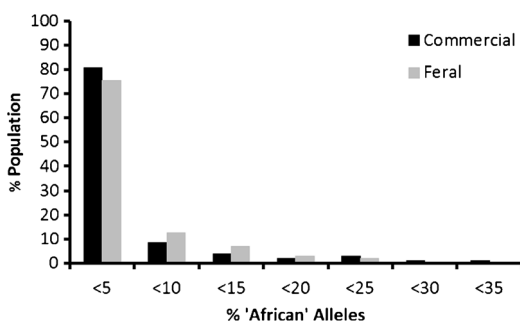


Figure 4. Proportion of the commercial and feral populations with African ancestry, in 5 % increments.

SNPs. AZ and BAH provided data. BPO, NCC, MHA and TER provided samples. NCC and BPO wrote the paper.

Origines hybrides des abeille australiennes (*Apis mellifera*)

Australie / apiculture / polymorphisme de nucléotide simple / sous-espèce / hybridation

Australische Honigbienen (*Apis mellifera*) sind gemischter Herkunft

Australien / Bienenhaltung / Single Nucleotide Polymorphismus / Unterarten der Honigbiene / Hybriden

REFERENCES

- Ajmone-Marsan, P., GLOBALDIV Consortium (2010) A global view of livestock biodiversity and conservation - GLOBALDIV. *Anim. Genet.* **41** (Supplementary 1), 1–5
- Alburaki, M., Bertrand, B., Legout, H., Moulin, S., Alburaki, A., Sheppard, W.S., Garnery, L. (2013) A fifth major genetic group among honeybees revealed in Syria. *BMC Genet.* **14**, 117
- Alburaki, M., Moulin, S., Legout, H., Alburaki, A., Garnery, L. (2011) Mitochondrial structure of Eastern honey bee populations from Syria, Lebanon and Iraq. *Apidologie* **42**, 628–641
- Cale, G.H., Gowen, J.W. (1956) Heterosis in the honey bee (*Apis mellifera* L.). *Genetics* **41**, 292–303
- Chapman, N.C., Harpur, B.A., Lim, J., Rinderer, T.E., Allsopp, M., Zayed, A., Oldroyd, B.P. (2015) A SNP test to identify Africanized honeybees via proportion of 'African' ancestry. *Mol. Ecol. Resour.* doi:10.1111/1755-0998.12411
- Chapman, N.C., Lim, J., Oldroyd, B.P. (2008) Population genetics of commercial and feral honey bees in Western Australia. *J. Econ. Entomol.* **101**, 272–277
- Chapman, N.C., Oldroyd, B.P., Hughes, W.O.H. (2007) Differential responses of honeybee (*Apis mellifera*) patrines to changes in stimuli for the generalist tasks of nursing and foraging. *Behav. Ecol. Sociobiol.* **61**, 1185–1194
- Chavez-Galarza, J., Henriques, D., Johnston, J.S., Azevedo, J.C., Patton, J.C., Munoz, I., de la Rua, P., Pinto, M.A. (2013) Signatures of selection in the Iberian honey bee (*Apis mellifera iberiensis*) revealed by a genome scan analysis of single nucleotide polymorphisms. *Mol. Ecol.* **22**, 5890–5907
- Coleman, R.S. (1956) Beekeeping in Western Australia - some historical notes. *J. Agr. West Aust.* **5**, 6
- De la Rua, P., Jaffe, R., Dall'Olio, R., Munzos, I., Serrana, J. (2009) Biodiversity, conservation and current threats to European honeybees. *Apidologie* **40**, 263–284
- De la Rua, P., Jaffe, R., Munoz, I., Serrano, J., Moritz, R.F.A., Kraus, F.B. (2013) Conserving genetic diversity in the honeybee: Comments on Harpur *et al.* (2012). *Mol. Ecol.* **22**, 3208–3210
- Dietemann, V., Pirk, C.W.W., Crewe, R. (2009) Is there a need for conservation of honeybees in Africa? *Apidologie* **40**, 285–295
- Eckert, J.E. (1958) The Kangaroo Island Ligurian bees. *Gleanings in Bee Culture* **86** (660–663), 722–725
- Franck, P., Garnery, L., Loiseau, A., Oldroyd, B.P., Hepburn, H.R., Solignac, M., Cornuet, J.M. (2001) Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. *Heredity* **86**, 420–430
- Franck, P., Garnery, L., Solignac, M., Cornuet, J.M. (2000) Molecular confirmation of a fourth lineage in honeybees from the Near East. *Apidologie* **31**, 167–180
- Garnery, L., Cornuet, J.-M., Solignac, M. (1992) Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. *Mol. Ecol.* **1**, 145–154
- Goodacre W.A. (1935) The beginner in bee culture. In: *Farmer's Bulletin*, p. 91. Department of Agriculture New South Wales, Sydney, Australia.
- Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Finlay, E.K., Jianlin, H., Groeneveld, E., Weigend, S., GLOBALDIV Consortium (2010) Genetic diversity in farm animals - a review. *Anim. Genet.* **41** (Supplement 1), 6–31
- Gulliford, R. (2005) Beekeeping in Australia. The Australasian Beekeeper, Rutherford, Australia
- Hall, S.J.G. (2004) Livestock Biodiversity. Genetic Resources for the Farming of the Future. Blackwell Science, Oxford, UK
- Harpur, B.A., Kent, C.F., Molodtsova, D., Lebon, J.M.D., Alqarni, A.S., Owayss, A.A., Zayed, A. (2014) Population genomics of the honey bee reveals strong signatures of positive selection on worker traits. *Proc. Natl. Acad. Sci. USA* **111**, 2614–2619
- Harpur, B.A., Minaei, S., Kent, C.F., Zayed, A. (2012) Management increases genetic diversity of honey bees via admixture. *Mol. Ecol.* **21**, 4414–4421
- Harpur, B.A., Minaei, S., Kent, C.F., Zayed, A. (2013) Admixture increases diversity in managed honey bees: Reply to De la Rua *et al.* (2013). *Mol. Ecol.* **22**, 3211–3215
- Harrison, J.F., Fewell, J.H., Anderson, K.E., Loper, G.M. (2006) Environmental physiology of the invasion of the Americas by Africanized honeybees. *Integr. Comp. Biol.* **46**, 1110–1122
- Hopkins, I. (1886) Illustrated Australasian Bee Manual and Complete Guide to Modern Bee Culture in the Southern Hemisphere, 3rd edn. Issac Hopkins, Auckland, New Zealand

- Jensen, A.B., Palmer, K.A., Boomsma, J.J., Pedersen, B.V. (2005) Varying degrees of *Apis mellifera ligustica* introgression in protected populations of the back honeybee, *Apis mellifera mellifera*, in northwest Europe. *Mol. Ecol.* **14**, 93–106
- Jensen, A.B., Pedersen, B.V. (2005) Honeybee conservation: A case story from Laeso Island, Denmark. In: Lodesani, M., Costa, C. (eds.) *Beekeeping and conserving biodiversity of honeybees*, pp. 142–164. Northern Bee Books, Hebden Bridge UK
- Jones, J.C., Myerscough, M.R., Graham, S., Oldroyd, B.P. (2004) Honey bee nest thermoregulation: Diversity promotes stability. *Science* **305**, 402–404
- Keogh, R.C., Robinson, A.P.W., Mullins, I.J. (2010) *Pollination Aware: The Real Value of Pollination in Australia*. Rural Industries Research and Development Corporation, Canberra, Australia
- Klein, A.M., Vaissiere, 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. *P. Roy. Soc. B. Biol. Sci.* **274**, 303–313
- Koulianos, S., Crozier, R. (1996) Mitochondrial DNA sequence data provides further evidence that the honeybees of Kangaroo Island, Australia are of hybrid origin. *Apidologie* **27**, 165–174
- Koulianos, S., Crozier, R. (1997) Mitochondrial sequence characterisation of Australian commercial and feral honeybee strains, *Apis mellifera* L. (Hymenoptera: Apidae), in the context of the species worldwide. *Aust. J. Entomol.* **36**, 359–363
- Lenstra, J.A., Groeneveld, L.F., Eding, H., Kantanen, J., Williams, J.L., Taberlet, P., Nicolazzi, E.L., Solkner, J., Simianer, H., Ciani, E., Garcia, J.R., Bruford, M.W., Ajmone-Marsan, P., Weigend, S. (2012) Molecular tools and analytical approaches for the characterization of farm animal genetic diversity. *Anim. Genet.* **43**, 483–502
- Mattila, H.R., Seeley, T.D. (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317**, 362
- Meixner, M.D., Costa, C., Kryger, P., Hatjina, F., Bouga, M., Ivanova, E., Buchler, R. (2010) Conserving diversity and vitality for honey bee breeding. *J. Apic. Res.* **49**, 85–92
- Oldroyd, B.P., Cornuet, J.M., Rowe, D., Rinderer, T.E., Crozier, R.H. (1995) Racial admixture of *Apis mellifera* in Tasmania, Australia - similarities and differences with natural hybrid zones in Europe. *Heredity* **74**, 315–325
- Oldroyd, B.P., Fewell, J.H. (2007) Genetic diversity promotes homeostasis in insect colonies. *Trends Ecol. Evol.* **22**, 408–413
- Oldroyd, B.P., Moran, C. (1987) Additive and heterotic genetic effects in the haplo-diploid honeybee *Apis mellifera*. *Aust. J. Bio. Sci.* **40**, 57–63
- Oldroyd, B.P., Moran, C., Nicholas, F.W. (1985) Diallele crosses of honeybees 1. A genetic-analysis of honey production using a fixed effects model. *J. Apic. Res.* **24**, 243–249
- Oldroyd, B.P., Moran, C., Nicholas, F.W. (1987) Diallele crosses of honeybees 2. A note presenting an estimate of the heritability of honey production under Australian conditions. *Aust. J. Agric. Res.* **38**, 651–654
- Oldroyd, B.P., Rinderer, T.E., Harbo, J.R., Buco, S.M. (1992) Effects of intracolony genetic diversity of honey bee (Hymenoptera, Apidae) colony performance. *Ann. Entomol. Soc. Am.* **85**, 335–343
- Oldroyd, B.P., Sheppard, W.S., Stelzer, J.A. (1993) Genetic characterization of the bees of Kangaroo Island, South Australia. *J. Apic. Res.* **31**, 141–148
- Oleska, A., Chybicki, I., Tofilski, A., Burczyk, J. (2011) Nuclear and mitochondrial patterns of introgression into native dark bees (*Apis mellifera mellifera*) in Poland. *J. Apic. Res.* **50**, 116–129
- Oxley, P.R., Oldroyd, B.P. (2009) Mitochondrial sequencing reveals five separate origins of 'Black' *Apis mellifera* (Hymenoptera: Apidae) in Eastern Australian commercial colonies. *J. Econ. Entomol.* **102**, 480–484
- Palmer, K.A., Oldroyd, B.P. (2003) Evidence for intra-colonial genetic variance in resistance to American foulbrood of honey bees (*Apis mellifera*): Further support for the parasite/pathogen hypothesis for the evolution of polyandry. *Naturwissenschaften* **90**, 265–268
- Pinto, M.A., Henriques, D., Chaves-Galarza, J., Kryger, P., Garnery, L., Van der Zee, M., Dahle, B., Soland-Reckeweg, G., de la Rua, P., Dall'Olio, R., Carreck, N.L., Johnson, J.S. (2014) Genetic integrity of the Dark European honey bee (*Apis mellifera mellifera*) from protected populations: A genome-wide assessment using SNPs and mtDNA sequence data. *J. Apic. Res.* **53**, 269–278
- Pritchard, J.K., Stephens, M., Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959
- Pritchard J.K., Wen X., Falush D. (2010) Documentation for *structure* software: Version 2.3, p. 7, http://pritchardlab.stanford.edu/structure_software/release_versions/v2.3.4/structure_doc.pdf.
- Rhodes J.W. (2011) *Quality of commercially reared queen and drone honey bees (Apis mellifera L.) in eastern Australia*, University of Western Sydney, Australia.
- Ruttner, F. (1976) Isolated populations of honeybees in Australia. *J. Apic. Res.* **15**, 97–104
- Ruttner, F. (1988) *Biogeography and Taxonomy of Honeybees*. Springer, Berlin, Germany
- Sambrook, J., Fritsch, E.F., Maniatis, T. (1989) *Molecular cloning*, 2nd edn. Cold Spring Harbor Laboratory Press, New York, USA
- Schiff, N.M., Sheppard, W.S., Loper, G.M., Shimanuki, H. (1994) Genetic diversity of feral honey bee (Hymenoptera: Apidae) populations in the southern United States. *Ann. Entomol. Soc. Am.* **87**, 842–848
- Schneider, S.S., DeGrandi-Hoffman, G., Smith, D.R. (2004) The African honey bee: factors contributing to a successful biological invasion. *Annu. Rev. Entomol.* **49**, 351–376

- Seeley, T.D. (1985) Honeybee Ecology: A Study of Adaptation in Social Life. Princeton University Press, Princeton, USA
- Seeley, T.D., Tarpy, D.R. (2007) Queen promiscuity lowers disease within honeybee colonies. *P. Roy. Soc. B Bio. Sci.* **274**, 67–72
- Strange, J.P., Gamery, L., Sheppard, W.S. (2007) Persistence of the Landes ecotype of *Apis mellifera mellifera* in southwest France: confirmation of a locally adaptive annual brood cycle trait. *Apidologie* **38**, 259–267
- Tarpy, D.R. (2003) Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. *P. Roy. Soc. B Biol. Sci.* **270**, 99–103
- Tarpy, D.R., Seeley, T.D. (2006) Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs. monandrous queens. *Naturwissenschaften* **93**, 195–199
- Tarpy, D.R., van Engelsdorp, D., Pettis, J.S. (2013) Genetic diversity affects colony survivorship in commercial honey bee colonies. *Naturwissenschaften* **100**, 723–728
- Wallberg, A., Han, F., Wellhagen, G., Dahle, B., Kawata, M., Haddad, N., Simoes, Z.L.P., Allsopp, M.H., Kandemir, I., de la Rua, P., Pirk, C.W., Webster, M.T. (2014) A worldwide survey of genome sequence variation provides insight into the evolutionary history of the honeybee *Apis mellifera*. *Nature Genet.* **48**, 1081–1088
- Weatherhead T. (1986) Boxes to Bar Hives: Beekeeping History of Queensland. International Colour Productions.
- 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.B., Tsutsui, N.D. (2006) Thrive out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. *Science* **314**, 642–645
- Winston, M.L. (1987) The Biology of the Honey Bee. Harvard University Press, Cambridge
- Woodward, D. (1993) Ligurian bees. *Am. Bee J.* **133**, 124–125