Author's personal copy

Journal of Insect Physiology 58 (2012) 402–407

Contents lists available at SciVerse ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Within- and across-colony effects of hyperpolyandry on immune function and body condition in honey bees (*Apis mellifera*)

Noah Wilson-Rich [a,1], David R. Tarpy [b,c,*], Philip T. Starks [a]

[a] Department of Biology, Tufts University, Medford, MA 02155, United States
[b] Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613, United States
[c] W.M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, NC 27695-7613, United States

ARTICLE INFO

Article history:
Received 12 November 2011
Received in revised form 27 December 2011
Accepted 28 December 2011
Available online 4 January 2012

Keywords:
Social insects
Genetic diversity
Polyandry
Immune response

ABSTRACT

Honey bees (*Apis mellifera*) have become a model system for studies on the influence of genetic diversity on disease. Honey bee queens mate with a remarkably high number of males—up to 29 in the current study—from which they produce a colony of genetically diverse daughter workers. Recent evidence suggests a significant benefit of intracolony genetic diversity on disease resistance. Here, we explored the relationship between the level of genetic diversity and multiple physiological mechanisms of cellular and humoral immune defense (encapsulation response and phenoloxidase activity). We also investigated an effect of genetic diversity on a measure of body condition (fat body mass). While we predicted that mean colony phenoloxidase activity, encapsulation response, and fat body mass would show a positive relationship with increased intracolonial genetic diversity, we found no significant relationship between genetic diversity and these immune measures, and found no consistent effect on body condition. These results suggest that high genetic diversity as a result of extreme polyandry may have little bearing on the physiological mechanisms of immune function at naturally occurring mating levels in honey bees.
© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Insect societies have long been used for evolutionary study of cooperation and conflict (reviewed by Ratnieks et al., 2006). Hamilton's inequality (Hamilton, 1964) addressed Darwin's concern of sterile workers within the eusocial Hymenoptera and explained how reproductive altruism could be beneficial among related individuals (but see Nowak et al., 2010). Kin selection theory, as applied to eusocial insects, explains that for workers to provide assistance, the benefits of performing a behavior should outweigh the costs after being devalued by the level of relatedness between the actor and the recipient (rB > C). While high levels of relatedness seem necessary for this to occur, this is often not observed across colonies within the eusocial Hymenoptera (Crozier and Pamilo, 1996).

Haplodiploidy is exhibited in many social insect systems, including the entire insect order Hymenoptera, whereby males are haploid and females are diploid. Such a genetic system therefore establishes asymmetries in relatedness among individuals within a colony, where daughter workers are related to each other by $r = 0.75$ if they share the same mother and father (supersisters, in the same patriline), but are only related to each other by $r = 0.25$ if they share the same mother and different fathers (half sisters, in different patrilines). Because mean colony relatedness decreases when multiple patrilines are present, increased levels of polyandry (multiple mating by queens) have long been seen as inconsistent with kin selection theory.

While monandry is largely prevalent among the social Hymenoptera (Keller and Reeve, 1994; Strassmann, 2001), polyandry is fairly widespread but unusual, with only a few extreme cases (i.e., hyperpolyandry; Crozier and Fjerdingstad, 2001) exclusive to highly social species. In honey bees (genus *Apis*), queens are known to mate with many males, with colonies consisting of an average of approximately 12 subfamilies depending on the species (Tarpy et al., 2004). Given what we know about kin selection theory and queen–worker conflict, the phenomenon of polyandry in honey bee colonies is therefore puzzling. Mean colony relatedness does not effectively decrease after approximately 10 successful matings; there is an asymptote in this relationship (Rueppell et al., 2008). It has been suggested that the adaptive benefit of polyandry up to this threshold likely originated as a mechanism to avoid the production of sterile, diploid male honey bees (Page, 1980; Ratnieks, 1990; Tarpy and Page, 2002) but that other (non-mutually exclusive) advantages are largely responsible for more extreme levels of hyperpolyandry (Palmer and Oldroyd, 2000; Rueppell et al., 2008).

* Corresponding author at: Department of Entomology, North Carolina State University, Raleigh, NC 27695-7613, United States. Tel.: +1 919 515 1660; fax: +1 919 515 7746.
E-mail addresses: noah@bestbees.com (N. Wilson-Rich), david_tarpy@ncsu.edu (D.R. Tarpy), philip.starks@tufts.edu (P.T. Starks).
[1] Present address: Best Bees Company, Boston, MA 02118, United States.

0022-1910/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jinsphys.2011.12.020

Author's personal copy

Recent data show that hyperpolyandry in honey bees provides a measure of disease resistance to the colony (Palmer and Oldroyd, 2003; Seeley and Tarpy, 2007; Tarpy, 2003; Tarpy and Seeley, 2006). Tarpy (2003) showed that colonies reared from queens inseminated from 24 drones had lower variance in their ability to resist *Ascosphaera apis*, the fungal pathogen that causes chalkbrood disease in honey bee brood, as compared to queens inseminated by only one drone. This observation indicates that multiple matings increase the likelihood that a colony will survive chalkbrood disease, whereas a colony headed by a monogamous queen may be more prone to a sweeping infection. Tarpy and Seeley (2006) followed this study by setting up colonies headed by queens inseminated with either one or 10 drones. They found that colonies with higher genetic diversity had lower intensity of all measured brood diseases (chalkbrood, sacbrood, American foulbrood, and European foulbrood). This observation was confirmed after artificial infection with the bacterium *Paenibacillus larvae*, the causative agent of the highly virulent American foulbrood disease (Seeley and Tarpy, 2007); colonies headed by queens inseminated with sperm from 10 drones had lower disease intensity and higher colony strength (i.e., more brood, heavier, and more populous). In each of these studies, however, only colony-level phenotypes were measured, and thus it is unclear as to what mechanism(s) lead to disease tolerance.

In this study, we aimed to determine the influence of colony-level genetic diversity on two standard immune measures—one constitutive (phenoloxidase activity, or PO activity) and one inducible (encapsulation response)—that could be responsible for the recent observations linking genetic diversity to disease resistance. We hypothesized that immune function explains the results we see in genetically diverse versus genetically uniform colonies. With recent advances in our understanding of honey bee immunity (Evans et al., 2006; Wilson-Rich et al., 2008; reviewed in Wilson-Rich et al., 2009), we can test physiological defense mechanisms by exploring the earliest cellular and humoral immune mechanisms that pathogens and parasites encounter once in the honey bee hemocoel. We predict a positive relationship between level of genetic diversity (number of patrilines) and both immune function (phenoloxidase activity and encapsulation response) and body condition (fat body mass). We examined these factors both among colonies (by comparing number of effective patrilines) and within colonies (by comparing among patrilines). Specifically, we predict that as genetic diversity increases, the mean immune function will also increase across colonies, while the variation around the mean will decrease (see Sherman et al., 1988). Within colonies, we predict a patriline effect on immune function and body condition, whereby different sibling groups will vary from each other.

2. Materials and methods

2.1. Specimen collection

1124 honey bees were collected from 22 colonies at the Cummings School of Veterinary Medicine at Tufts University in North Grafton, MA, USA during two non-consecutive years. In 2006, guarding and foraging adult workers were collected from 12 colonies (N = 266 individuals, mean ± SD = 22.17 ± 5.18 individuals per colony, range 12–28). In 2009, guarding and foraging adults, as well as brood, were collected from 10 colonies (N = 858 individuals total, mean ± SD = 85.80 ± 8.09 individuals per colony, range 66–95 per colony). Brood collection involved mixed samples of larvae and pupae, as the immune function measures used (phenoloxidase activity and encapsulation response) are the same across both developmental stages (Wilson-Rich et al., 2008).

2.2. Immunology and body condition

Phenoloxidase activity (PO), encapsulation response (ER), and fat body mass (FB) were performed following the methods described by Wilson-Rich et al. (2008). Briefly, PO activity (a constitutive measure of immune function with no previous challenge) was measured in samples of bee hemolymph extracted from tested bees. Encapsulation response was quantified after implanting a nylon thread in adult abdomens and by using image analysis software. Finally, fat body mass was measured as the percent change in abdominal weight after an ethyl ether wash. In 2006, 133 adults were assayed for ER and 310 adults for FB. In 2009, 256 individual brood were assayed for PO and 299 adults for FB. Not all individuals collected produced usable data based on limitations of each respective assay, such as inability to collect hemolymph for PO or failure to retrieve the implant for ER. A highly conservative threshold was implemented for PO analyses, whereby the Vmax of enzyme linear phases were used only if $r^2 \geqslant 0.9$. Multiple measures were conducted in an effort to gain more information about the effects of genetic diversity across the dynamic immune system (see Adamo, 2004). We assessed two measures (ER, PO) of immune function and one measure of body condition (FB) for a holistic view of immunocompetence (see Wilson-Rich et al., 2009).

2.3. Genetic analyses

Colony-level genetic diversity and within-colony patrilines were quantified blindly after samples were collected for immune tests. The subfamily of each individual was determined using polymorphic microsatellite genetic analysis (c.f., Delaney et al., 2011; Tarpy et al., 2010). Because of their generally higher mutation rates, microsatellite markers may have large numbers of alleles, which make them particularly suited for paternity analysis (Estoup et al., 1995, 1994). DNA was extracted from all 1124 individuals (N = 266 adults from the 2006 collection and N = 858 brood from the 2009 collection) using Chelex® 100 (Walsh et al., 1991) and subject to PCR at eight microsatellite loci following Delaney et al. (2011). Paternity was then assigned to each worker following standard methods (Estoup et al., 1994), and the observed mating number and effective paternity frequency was calculated for each queen following Nielsen et al. (2003).

2.4. Statistical analyses

Immune function was compared both across and within colonies based on the number of patrilines, and patriline of origin, respectively. For across colony comparisons, we created a univariate generalized linear model (GLM) to control for any effect of subfamily differences within colonies. The GLM ANOVA incorporated each respective immune measure as discrete dependent variables, the number of subfamilies in each respective colony as the independent variable, and patriline as a covariate nested in each colony. We did not analyze genetics from adults in 2009 (only brood genetics were conducted for the 2009 samples), so these individuals were compared separately from the GLM, and Kruskal–Wallis H tests were performed instead. For within-colony comparisons, we first determined whether each data set was normally distributed using Shapiro–Wilk tests. We then performed either ANOVAs or Kruskal–Wallis H tests across patrilines followed by post hoc pairwise comparisons, depending upon the normality of the distribution of each respective data set from Levene's tests. Comparisons of variation were conducted also using Levene's tests for unequal variance. Furthermore, we calculated partial eta-squared values (as in Wilson-Rich et al., 2008) to determine the proportion of variance in each immune measure that may be ex-

Author's personal copy

plained by colony-level genetic diversity. All statistics were calculated using SPSS for Windows (v. 11).

3. Results

3.1. Genetics

Across the 22 colonies, we found a range of 8–29 patrilines per colony (mean ± SD: 15.4 ± 6.3; Table 1). In 2006, the number of patrilines ranged from 9 to 15 (mean ± SD: 11.0 ± 2.3), while in 2009 the number of patrilines ranged from 11 to 29 (mean ± SD: 20.8 ± 5.2). As is typical for colonies headed by naturally mated queens, the representation of drone fathers among the worker offspring was not equal, leading to effective paternity frequencies well within the normal range of the species (mean ± SD: 13.4 ± 5.8).

3.2. Across-colony comparisons

A total of 998 honey bees were used for immune function tests. We tested the ER on 133 adult bees taken from six colonies in 2006 (mean ± SD = 21.16 ± 4.40 bees per colony). Overall, the number of subfamilies was not a significant factor contributing to ER ($F = 0.35$, $df = 5$, $P > 0.5$; Fig. 1). Variation around the average ER differed significantly with number of subfamilies ($F = 2.01$, $df = 62$, 64, $P < 0.01$). The number of subfamilies contributed remarkably little to predicting ER (eta-squared = 0.092).

We assayed PO on brood from seven colonies in 2009 ($N = 229$ individuals, mean ± SD = 32.71 ± 9.40 bees per colony). Overall, the number of subfamilies was a significant factor contributing to PO ($F = 7.91$, $df = 6$, $P < 0.001$; Fig. 2). Variation around the average PO also differed significantly with number of subfamilies ($F = 1.68$, $df = 96$, 132, $P < 0.01$). Post hoc pairwise comparisons of colony median and variance revealed haphazard relationships, with no clear directional trend for how patriline number influences PO activity (Fig. 2). The number of subfamilies in a honey bee hive contributed to approximately 1/2 of the predictive value determining PO mass (eta-squared = 0.498).

We quantified FB on 264 adults from 12 colonies in 2006 (mean ± SD = 22.00 ± 5.34 bees per colony). Overall, the number of subfamilies was a significant factor contributing to FB ($F = 9.70$, $df = 11$, $P < 0.001$; Fig. 3). Variation around the average FB also differed significantly with number of subfamilies ($F = 1.92$, $df = 130$, 33, $P < 0.001$). In 2009, 266 adults from 10 colonies were assayed for FB (mean ± SD = 26.60 ± 7.73 bees per colony). Again, the number of subfamilies was a significant factor ($H = 17.97$, $df = 9$, $P = 0.05$). The number of subfamilies in a honey bee hive contributed to just over 2/3 of the predictive value determining FB mass (eta-squared = 0.680).

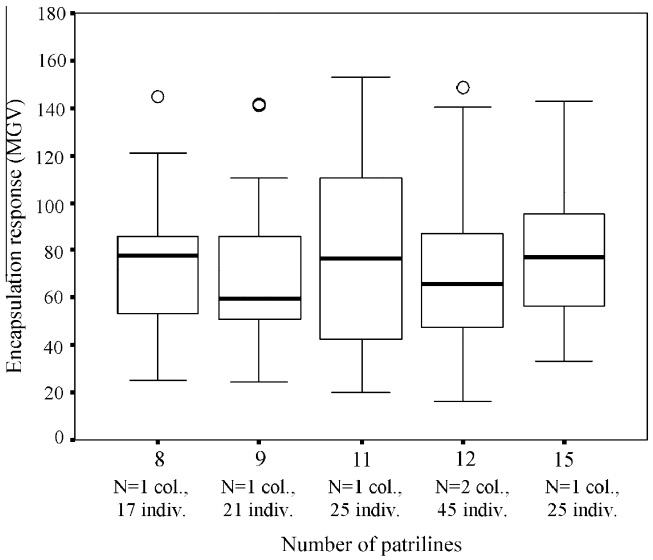

Fig. 1. Mean colony encapsulation response does not correlate with increasing number of patrilines in honey bees (*A. mellifera*). Boxes represent middle 50% of data, lines in boxes show median values, and whiskers represent 95% of data, beyond which outliers reside. Circles show outliers. Sample sizes are noted along the *x*-axis as the number of individuals in each colony with representative number of patrilines stated beneath it.

Table 1
Mating number trends for honey bee (*A. mellifera*) queens. These data are an overview of genetic results from 22 honey bee colonies at the Tufts University apiary in North Grafton, MA, USA. The number of patrilines for each colony was determined using the methods of Delaney et al. (2011). Overall, hives averaged 16.5 ± 6.3 patrilines, although it is likely that the estimated number of patrilines is subject to high variation based on our limited sample size (Tarpy and Nielsen, 2002).

Year	Number of bees collected per colony (n)	Number of subfamilies (N_0)	Number of bees per patriline (mean ± SD)
2006	18	9	2.00 ± 1.32
2006	28	15	1.87 ± 0.64
2006	22	12	1.83 ± 1.19
2006	20	8	2.50 ± 2.00
2006	25	11	2.27 ± 1.56
2006	27	12	2.25 ± 2.30
2006	27	15	1.80 ± 1.08
2006	18	11	1.64 ± 0.81
2006	17	12	1.42 ± 0.67
2006	28	9	3.11 ± 2.26
2006	24	9	2.67 ± 3.04
2006	12	9	1.33 ± 0.71
2009	85	11	7.73 ± 7.38
2009	85	29	2.93 ± 1.96
2009	84	25	3.36 ± 2.46
2009	84	17	4.94 ± 3.11
2009	91	23	3.96 ± 2.75
2009	95	17	5.59 ± 5.47
2009	94	25	3.76 ± 4.47
2009	89	23	3.87 ± 3.05
2009	66	20	3.30 ± 4.01
2009	85	18	4.72 ± 3.48

3.3. Within-colony comparisons

Looking within each colony, we compared each measure of immune function across subfamilies. There was no significant patriline effect on either ER or PO, in any of the colonies (ANOVA $P > 0.05$ or KW $P > 0.05$). We did, however, detect an effect of patriline in the FB analysis in about half of the colonies (2/12 colonies: colony F, $F = 2.52$, $df = 26$, $P = 0.05$; colony K, $F = 3.33$, $df = 8$, 15; $P < 0.05$).

4. Discussion

We found no evidence that high levels of intracolony genetic diversity affects either of the immune function parameters measured (Table 2). Remarkably, patriline of origin was not a predictor of either PO or ER in any of the 22 colonies analyzed despite known effects of genotype on the prevalence of disease within colonies (e.g., Evans, 2004). There was an effect of subfamily number on body condition within only 20% of the colonies. There was a significant patriline effect on fat body mass in two colonies. Fat bodies are vitally important for honey bees for energy and production of antimicrobial proteins (Hetru et al., 1998; Hultmark, 1993; Zachary and Hoffman, 1984).

Author's personal copy

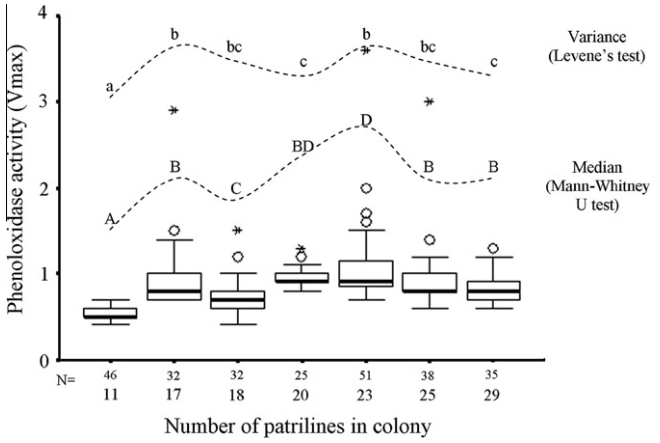

Fig. 2. Mean colony phenoloxidase activity does not correlate with increasing number of patrilines in honey bees (*A. mellifera*). Different letters indicate different medians and variances identified through pair-wise comparisons. Lines above boxes suggest trends across data sets for medians and variations around the median. Boxes represent middle 50% of data, lines in boxes show median values, and whiskers represent 95% of data, beyond which outliers reside. Circles show mild outliers and asterisks show extreme outliers. Sample sizes are noted along the *x*-axis as the number of individuals in each colony with representative number of patrilines stated beneath it.

We do not provide evidence that the average colony phenotype of these physiological mechanisms is influenced by intracolony genetic diversity (although other immune mechanisms, such as the production of antimicrobial peptides, may still be affected). The immune systems we studied, PO and ER, are important lines of defense to host insects after a pathogen enters the host hemocoel (reviewed in Wilson-Rich et al., 2009). We know that honey bee colonies reared from hyperpolyandrous queens have greater relative fitness against infectious disease compared to monandrous queens (Seeley and Tarpy, 2007; Tarpy, 2003; Tarpy and Seeley, 2006). The mechanism of disease resistance, therefore, remains unclear, but it is likely that a behavioral mechanism (e.g., hygienic behavior; Spivak and Reuter, 2001) may be responsible for such patterns in colony phenotype. These and other mechanisms of such 'social immunity' (sensu Cremer et al., 2007) should be investigated to gain an advanced understanding of how colony-level genetic diversity increases fitness by reducing disease.

Genetic diversity possibly provides more lines of defense against pathogens and parasites, assuming that more, different alleles coding for disease resistance phenotypes are entering the population at large. These additional and different defenses likely keep disease intensity in check (see Hughes and Boomsma, 2006). It is perhaps surprising that encapsulation response and phenoloxidase activity are not affected by colony-level genetic diversity, but it may be because these innate mechanisms may be too important for fighting pathogens and parasites. In other words, it is likely that their vital importance makes them indispensible to all individuals and therefore do not show variation. The one mode of immune defense with the most flexibility in phenotype is, of course, behavior.

Behavior is the most obvious mechanism for how genetic diversity may scale up to disease resistance, and as such, requires future study. Age polyethism is also influenced by patriline; honey bee colonies benefit from multiple subfamilies because the genotypes of workers affect the probabilities of initiating and ending behavior associated with colony division of labor (Calderone and Page, 1988; Frumhoff and Schneider, 1987; Mattila et al., 2008; Page et al., 1989; Robinson and Page, 1989). This relationship between patriline number and colony fitness should then scale up to result in hives with increased genetic diversity better withstand disease, assuming patriline also affects immune function.

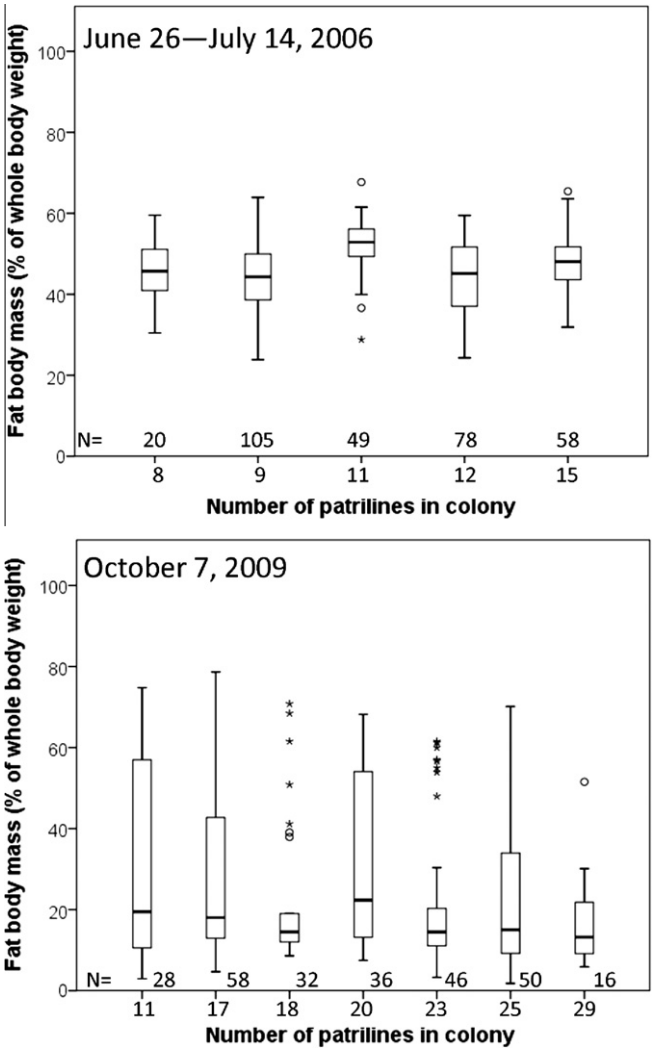

Fig. 3. Average colony fat body mass does not correlate with increasing number of patrilines in honey bees (*A. mellifera*). Boxes represent middle 50% of data, lines in boxes show median values, and whiskers represent 95% of data, beyond which outliers reside. Circles show outliers and asterisks show extremes. *N* indicates the number of analyzed bees.

Ultimately, the high variation in queen mating number in honey bees may be the result of queen's inability to assess the number of times they mate, which may result in extreme final mating numbers (Tarpy and Page, 2001; but see Schluns et al., 2005). As such, queens may have been selected to mate with a minimum number of drones but drones above the minimum are inconsequential. Alternate hypotheses for hyperpolyandry in honey bee queens include sperm limitation (Cole, 1983), sperm competition (Parker, 1984), and increased caste differentiation (Fuchs and Schade, 1994; Mattila and Seeley, 2007; Oldroyd and Fewell, 2007; Robinson, 1992; Rueppell et al., 2008). These hypotheses each explore mechanistic explanations for the benefits of a known adaptive behavior, and as such are not necessarily mutually exclusive.

Future tests may include examining induced immune effector molecules, either through studying their post-transcriptional (Manfredini et al., 2010a) or post-translational (Rosengaus et al., 2007) gene products. Constitutive and induced mechanisms of cellular immune function can be studied using advanced methods of differential hemocyte counts, types, and functional tests (Manfredini et al., 2010b, 2008). Subsequent experiments investigating constitutive and induced mechanisms of immunity will provide a better understanding of the evolution of disease resistance. On

Author's personal copy

Table 2
Summary of results testing the 'genetic diversity' hypothesis. We hypothesized that immune function explains the results seen in genetically diverse versus genetically monomorphic hives. Genetic diversity, although clearly a benefit to colony health, had no effect on these immune measures and no consistent effect on body condition.

Year	Immune function	Developmental stage	Hypothesis supported?	Significance
(1) Encapsulation response				
2006	Across colonies	Adults	No	No effect of patriline number
2006	Within colonies	Adults	No	No patriline effect
(2) Phenoloxidase activity				
2009	Across colonies	Brood	Unclear	Colony effect significant, although not correlated with patriline number (Fig. 2)
2009	Within colonies	Brood	No	No patriline effect
(3) Fat body mass				
2006 and 2009	Across colonies	Adults	No	No effect of patriline number
2006 and 2009	Within colonies	Adults	Yes, for 2/12 colonies	Weak patriline effect

the one hand, constitutive and innate mechanisms are highly conserved and therefore less variable in response to pathogen selection pressures over evolutionary time. On the other hand, induced and acquired mechanisms are less conserved, and so are expected to be more variable in quantity and quality. Additionally, actual disease resistance tests will provide valuable information about the actual biological relevance of each immune measure (Manfredini et al., 2010a).

Acknowledgements

Field colonies were organized with the help of Rick Reault and Augustus Skamarycz, and to them we are grateful. We would also like to thank Joel Caren for performing the genotyping of the colonies. Additionally, we appreciate Heather Mattila, Juliet Fuhrman, Sara Lewis, and Colin Orians for their helpful and valuable feedback on this manuscript. This project was supported with funding from Tufts to PTS and a Tufts Research Award to NWR, as well as by grant number 2007-02281 from the National Research Initiative of the USDA Cooperative State Research, Education and Extension Service awarded to DRT.

References

Adamo, S.A., 2004. How should behavioural ecologists interpret measurements of immunity? Animal Behaviour 68, 1443–1449.
Calderone, N.W., Page Jr., R.E., 1988. Genotypic variability in age polyethism and task specialization in the honey bee *Apis mellifera* (Hymenoptera: *Apidae*). Behavioral Ecology and Sociobiology 22, 17–26.
Cole, B.J., 1983. Multiple mating and the evolution of social behavior in the Hymenoptera. Behavioral Ecology and Sociobiology 12, 191–201.
Cremer, S., Armitage, S.A.O., Schmid-Hempel, P., 2007. Social immunity. Current Biology 17, R693–R702.
Crozier, R.H., Fjerdingstad, E.J., 2001. Polyandry in social Hymenoptera – disunity in diversity? Annales Zoologici Fennici 38, 267–285.
Crozier, R.H., Pamilo, P., 1996. Evolution of Social Insect Colonies: Sex Allocation and Kin Selection. Oxford University Press, New York.
Delaney, D.A., Keller, J.J., Caren, J.R., Tarpy, D.R., 2011. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera*). Apidologie 42, 1–13.
Estoup, A., Solignac, M., Cornuet, J., 1994. Precise assessment of the number of patrilines and of genetic relatedness in honeybee colonies. Proceedings of the Royal Society of London B 258, 1–7.
Estoup, A., Garnery, L., Solignac, M., Cornuet, J.-M., 1995. Microsatellite variation in honey bee (*Apis mellifera* L.) populations: hierarchical genetic structure and test of the infinite allele and stepwise mutation models. Genetics 140, 679–695.
Evans, J.D., 2004. Transcriptional immune responses by honey bee larvae during invasion by the bacterial pathogen, *Paenibacillus* larvae. Journal of Invertebrate Pathology 85, 105–111.
Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., Jiang, H., Kanost, M., Thompson, G.J., Zou, Z., Hultmark, D., 2006. Immune pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Molecular Biology 15, 645–656.
Frumhoff, P.C., Schneider, S., 1987. The social consequences of honey bee polyandry: the effects of kinship on worker interactions within colonies. Animal Behavior 35, 255–262.
Fuchs, S., Schade, V., 1994. Lower performance in honeybee colonies of uniform paternity. Apidologie 25, 155–168.
Hamilton, W.D., 1964. The genetical evolution of social behaviour I and II. Journal of Theoretical Biology 7, 1–52.
Hetru, C., Hoffmann, D., Bulet, P., 1998. Antimicrobial peptides from insects. In: Bulet, P., Hoffmann, D. (Eds.), Molecular Mechanisms of Immune Responses in Insects. Chapman and Hall, London, pp. 40–66.
Hughes, W.O.H., Boomsma, J.J., 2006. Does genetic diversity hinder parasite evolution in social insect colonies? Journal of Evolutionary Biology 19, 132–143.
Hultmark, D., 1993. Immune-reactions in Drosophila and other insects – a model for innate immunity. Trends in Genetics 9, 178–183.
Keller, L., Reeve, H.K., 1994. Genetic variability, queen number, and polyandry in social Hymenoptera. Evolution 48, 694–704.
Manfredini, F., Dallai, R., Ottaviani, E., 2008. Circulating hemocytes from larvae of the paper wasp *Polistes dominulus* (Hymenoptera, *Vespidae*). Tissue and Cell 40, 103–112.
Manfredini, F., Beani, L., Taormina, M., Vannini, L., 2010a. Parasitic infection protects wasp larvae against a bacterial challenge. Microbes and Infection 12, 727–735.
Manfredini, F., Benati, D., Beani, L., 2010b. The strepsipteran endoparasite Xenos vesparum alters the immunocompetence of its host, the paper wasp *Polistes dominulus*. Journal of Insect Physiology 56, 253–259.
Mattila, H.R., Seeley, T.D., 2007. Genetic diversity in honey bee colonies enhances productivity and fitness. Science 317, 362–364.
Mattila, H.R., Burke, K.M., Seeley, T.D., 2008. Genetic diversity within honeybee colonies increases signal production by waggle-dancing foragers. Proceedings of the Royal Society B: Biological Sciences 275, 809–816.
Nielsen, R., Tarpy, D.R., Reeve, H.K., 2003. Estimating effective paternity number in social insects and the effective number of alleles in a population. Molecular Ecology 12, 3157–3164.
Nowak, M.A., Tarnita, C.E., Wilson, E.O., 2010. The evolution of eusociality. Nature 466, 1057–1062.
Oldroyd, B.P., Fewell, J.H., 2007. Genetic diversity promotes homeostasis in insect colonies. Trends in Ecology and Evolution 22, 408–413.
Page Jr., R.E., 1980. The evolution of multiple mating behavior by honey bee queens (*Apis mellifera*). Genetics 96, 263–273.
Page Jr., R.E., Robinson, G.E., Fondrk, M.K., 1989. Genetic specialists, kin recognition and nepotism in honeybee colonies. Nature 339, 576–579.
Palmer, K.A., Oldroyd, B.P., 2000. Evolution of multiple mating in the genus *Apis*. Apidologie 31, 235–248.
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.
Parker, G.A., 1984. Sperm competition and the evolution of animal mating strategies. In: Smith, R.L. (Ed.), Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, FL, pp. 1–60.
Ratnieks, F.L.W., 1990. The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of removal of diploid males. Behavioral Ecology and Sociobiology 26, 343–348.
Ratnieks, F.L.W., Foster, K.R., Wenseleers, T., 2006. Conflict resolution in insect societies. Annual Review of Entomology 51, 581–608.
Robinson, G.E., 1992. Regulation of division-of-labor in insect societies. Annual Review of Entomology 37, 637–665.
Robinson, G.E., Page Jr., R.E., 1989. Genetic basis for division of labor in insect societies. In: Page, R.E., Jr. (Ed.), The Genetics of Social Evolution. Westview Press, Boulder, CO, pp. 61–80.
Rosengaus, R.B., Cornelisse, T., Guschanski, K., Traniello, J.F.A., 2007. Inducible immune proteins in the dampwood termite *Zootermopsis angusticollis*. Naturwissenschaften 94, 25–33.
Rueppell, O., Johnson, N., Rychtar, J., 2008. Variance-based selection may explain general mating patterns in social insects. Biology Letters 4, 270–273.
Schluns, H., Moritz, R.F.A., Neumann, P., Kryger, P., Koeniger, G., 2005. Multiple nuptial flights, sperm transfer and the evolution of extreme polyandry in honeybee queens. Animal Behaviour 70, 125–131.
Seeley, T.D., Tarpy, D.R., 2007. Queen promiscuity lowers disease within honeybee colonies. Proceedings of the Royal Society B: Biological Sciences 274, 67–72.
Sherman, P.W., Seeley, T.D., Reeve, H.K., 1988. Parasites, pathogens, and polyandry in social Hymenoptera. American Naturalist 131, 602–610.
Spivak, M., Reuter, G.S., 2001. Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. Apidologie 32, 555–565.

Author's personal copy

Strassmann, J.E., 2001. The rarity of multiple mating by females in the social Hymenoptera. Insectes Sociaux 48, 1–13.
Tarpy, D.R., 2003. Genetic diversity within honeybee colonies prevents severe infections and promotes colony growth. Proceedings of the Royal Society of London Series B: Biological Sciences 270, 99–103.
Tarpy, D.R., Nielsen, D.I., 2002. Sampling error, effective paternity, and estimating the genetic structure of honey bee colonies (Hymenoptera: *Apidae*). Annals of the Entomological Society of America 95, 513–528.
Tarpy, D.R., Page Jr., R.E., 2001. The curious promiscuity of queen honey bees (*Apis mellifera*): evolutionary and behavioral mechanisms. Annales Zoologici Fennici 38, 255–265.
Tarpy, D.R., Page Jr., R.E., 2002. Sex determination and the evolution of polyandry in honey bees (*Apis mellifera*). Behavioral Ecology and Sociobiology 52, 143–150.
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., Nielsen, R., Nielsen, D.I., 2004. A scientific note on the revised estimates of effective paternity frequency in *Apis*. Insectes Sociaux 51, 203–204.
Tarpy, D.R., Caren, J.R., Delaney, D.A., Sammataro, D., Finley, J., Loper, G.M., DeGrandi-Hoffman, G., 2010. Mating frequencies of Africanized honey bees in the south western USA. Journal of Apicultural Research 49, 302–310.
Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10, 506–513.
Wilson-Rich, N., Dres, S.T., Starks, P.T., 2008. The ontogeny of immunity: development of innate immune strength in the honey bee (*Apis mellifera*). Journal of Insect Physiology 54, 1392–1399.
Wilson-Rich, N., Spivak, M., Fefferman, N.H., Starks, P.T., 2009. Genetic, individual, and group facilitation of disease resistance in insect docieties. Annual Review of Entomology 54, 405–423.
Zachary, D., Hoffman, D., 1984. Lysozyme is stored in the granules of certain haemocyte types in Locusta. Journal of Insect Physiology 30, 405–411.