Pleiotropy of segregating genetic variants that affect honey bee worker life expectancy.

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Abstract:

In contrast to many other complex traits, the natural genetic architecture of life expectancy has not been intensely studied, particularly in non-model organisms, such as the honey bee (Apis mellifera L.). Multiple factors that determine honey bee worker lifespan have been identified and genetic analyses have been performed on some of those traits. Several of the traits are included in a suite of correlated traits that form the pollen hoarding syndrome, which was named after the behavior to store surplus pollen in the nest and is tied to social evolution. Here, seven quantitative trait loci that had previously been identified for their effects on different aspects of the pollen hoarding syndrome were studied for their genetic influence on the survival of adult honey bee workers. To gain a more comprehensive understanding of the genetic architecture of worker longevity, a panel of 280 additional SNP markers distributed across the genome was also tested. Allelic distributions were compared between young and old bees in two backcross populations of the bi-directionally selected high- and low-pollen hoarding strain. Our results suggest a pleiotropic effect of at least one of the behavioral quantitative trait loci on worker longevity and one significant and several other putative genetic effects in other genomic regions. At least one locus showed evidence for strong antagonistic pleiotropy and several others suggested genetic factors that influence pre-emergence survival of worker honey bees. Thus, the predicted association between worker lifespan and the pollen hoarding syndrome was supported at the genetic level and the magnitude of the identified effects also strengthened the view that naturally segregating genetic variation can have major effects on age-specific survival probability in the wild.

Keywords: longevity | lifespan | social evolution | division of labor | QTL | pleiotropy | genetic architecture | honey bees | social biology | gerontology | biology

Article:

1. Introduction

Complex traits that are commonly studied in a quantitative genetic context include behavior (Oldroyd and Thompson, 2007), disease (Lander and Schork, 1994), and many life history traits (Mackay, 2001). However, relatively few detailed studies of individual lifespan exist (Shmookler Reis et al., 2006), although this trait is probably the most complex trait (Finch and Tanzi, 1997 and Mackay, 2002), with strong genetic (Kenyon, 2005 and Wang et al., 2004) and environmental influences (Carey et al., 1998), interactions between loci (Leips and Mackay, 2000), interactions between genotype and environment (Paaby and Schmidt, 2009 and Shook and Johnson, 1999), and a large stochastic component (Finch and Kirkwood, 2000). Death, the ultimate determinant of lifespan, can have numerous causes, ranging from intrinsic, molecular causes to external, macro-environmental circumstances, and multiple interactions among the different factors (McGinnis and Foege, 1993).

The genetic architecture of lifespan has been investigated with quantitative trait loci (QTL) studies mostly in the model species Caenorhabditis elegans (Ayyadevara et al., 2003, Ebert et al., 1993 and Shook and Johnson, 1999), Drosophila (Lai et al., 2007, Leips and Mackay, 2000 and Paaby and Schmidt, 2009), and Mus musculus (Jackson et al., 2002 and Lang et al., 2010). A few studies also exist in humans (Perls and Terry, 2003). However, the genetic architecture of life expectancy or ongevity has not been characterized in many species. In contrast, significant progress has been made in understanding the molecular processes and genes that can potentially affect lifespan when experimentally manipulated (Shmookler Reis et al., 2006 and Slagboom et al., 2011).

The honey bee (Apis mellifera L.) is an emergent model organism for biogerontological research (Omholt and Amdam, 2004, Page and Peng, 2001 and Rueppell et al., 2004b). Social (Amdam et al., 2009, Rueppell et al., 2007 and Rueppell et al., 2008) and physiological (Nelson et al., 2007) variables that influence honey bee life expectancy have been identified. Most notably, the reproductive queen lives an order of magnitude longer than the non-reproductive workers, despite a similar genome and environment (Page and Peng, 2001). Pronounced variation in life expectancy also exists among workers, which has been related to the timing of their age-related specialization in different behavioral tasks (Nelson et al., 2007 and Rueppell et al., 2007).

The timing of the behavioral ontogeny in adult workers is related to the pollen hoarding syndrome, a life history syndrome that has been initially characterized in artificially selected strains but applies to honey bees in general (Page et al., 2007). The pollen hoarding syndrome

has been named after the propensity of worker bees to store surplus pollen in their nest. It comprises a number of correlated traits, including reproductive and sensory traits, learning and memory, and foraging behavior. For example, high pollen hoarding workers have larger ovaries, are more responsive to sucrose, learn faster, initiate foraging earlier, and specialize more in pollen collection than their low pollen hoarding counterparts (Page et al., 2007). The genetic architecture of multiple traits of the pollen hoarding syndrome has been characterized, revealing several significant QTL. Some of these QTL affect multiple traits, indicating partial overlap in the genetic determination of these traits due to pleiotropy (Graham et al., 2011, Rueppell et al., 2006, Rüppell et al., 2004 and Wang et al., 2009).

Seven of the identified QTL are of particular interest for aging research. The pollen hoarding QTL (pln1-4) were originally identified for their effect on foraging behavior of workers (Hunt et al., 1995, Page et al., 2000 and Rüppell et al., 2004) but show pleiotropic effects on sucrose responsiveness (Rueppell et al., 2006), ovary size (Graham et al., 2011 and Wang et al., 2009), and the timing of the irreversible transition of workers from in-hive tasks to foraging (Rueppell et al., 2004b), termed the age of first foraging. The pln QTL are significantly enriched for genes involved in insulin/insulin-like signaling (Hunt et al., 2007), which has been implicated in the endocrine regulation of aging in a number of species (Tatar et al., 2003). Two of these candidate genes have been directly implicated in the pollen hoarding syndrome (Wang et al., 2009). The other QTL of interest (aff2-4) have been identified for their effects on the worker age of first foraging (Rueppell, 2009 and Rueppell et al., 2004a). They are particularly relevant in the context of life expectancy because the age of first foraging is the strongest predictor of honey bee worker lifespan in all studies that have investigated its effect (Rueppell et al., 2007 and Rueppell et al., 2008).

Based on these findings, we predicted the pln and aff QTL to show an effect on honey bee worker life expectancy. We tested this prediction in reciprocal backcrosses between the high and low pollen hoarding strains. These strains have been artificially selected from commercial honey bees for the amount of pollen stored in their hive (Page and Fondrk, 1995). They show a number of correlated selection responses that originally defined the pollen hoarding syndrome (Page et al., 2007). These two strains also differ significantly in worker life expectancy (Amdam et al., 2007 and Rueppell et al., 2007). Therefore, we screened a panel of 280 random SNPs throughout the genome for their effect on worker life expectancy, in addition to conducting specific tests of the effect of the three aff and four pln QTL.

2. Methods and materials

2.1. Mapping populations

The high and low pollen hoarding strains that have been bi-directionally selected for the amount of pollen stored in their hive (Page and Fondrk, 1995) were used to set up our experimental crosses. The high and low pollen hoarding strains have been maintained by circular inbreeding with occasional outcrossing to phenotypically similar, but unrelated colonies because honey bees cannot be directly inbred due to their complementary sex determination mechanism (Beye et al., 2003). After 20 generations, reciprocal backcrosses were set up by artificial insemination of one low strain queen with the semen of one high strain male (Fig. 1). Resulting daughters were raised to queens and then either backcrossed to one high strain male (high backcross: HBC) or one low strain male (low backcross: LBC).

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The LBC and HBC queens were both caged on empty wax cells in their respective hives 21 days before the onset of the experiment. The resulting brood was transferred 1 day before emergence to an incubator (34 °C, 50% relative humidity). Small, random subsets of newly emerged LBC (n = 130) and HBC (n = 160) workers were frozen directly after emergence. The majority of LBC (n = 1300) and HBC (n = 1600) worker bees were color-marked on their thorax with non-toxic paint (Testors™) according to their LBC and HBC identity and introduced into one unrelated host hive. Survival of these cohorts was monitored nightly by counting all marked workers present in the hive. All surviving bees were collected at the age of 40 days, when ≤ 10% of the workers in both cohorts were alive. The host hive was opened at dusk when foraging activity had ceased and all marked bees were collected directly onto dry ice. Genomic DNA was extracted from the frozen worker samples, using a modified phenol-chloroform protocol (Hunt and Page, 1995). DNA quantity and quality were assessed using a Nanodrop spectrophotometer (Thermo Scientific, MA).

2.2. SNP genotyping

To search the honey bee genome for potential genetic factors that influence adult survival probability without a priori information, 20 random samples for each of the four groups (HBC young, HBC old, LBC young, LBC old) were genotyped at 280 single nucleotide polymorphisms (SNPs), that were selected from a larger set of confirmed SNPs (Whitfield et al., 2006). SNP genotyping was performed by Sequenom (CA), using MALDI-TOF (Matrix-assisted laser desorption/ionization-time-of-flight) mass spectrometry with automatic genotype calling (Ragoussis et al., 2006), according to internal company protocols. Loci with > 50% missing genotypes were omitted in the HBC (16 loci) and LBC (18 loci). One individual in the LBC was completely excluded because it resulted in erroneous genotypes in > 50 loci, indicating insufficient DNA quality. Monomorphic loci (HBC: 171, LBC: 170) were also excluded from the subsequent analyses because they were uninformative. For all informative, polymorphic

markers (93 in the HBC and 92 in the LBC) allele frequency differences between the young and the old cohorts were evaluated by a 2 × 2 contingency analysis, using Fisher's exact test to calculate the probability of an association between cohort age and genotype frequencies. A significant association indicated a shift of allele frequencies from young to old bees in a particular SNP and a linked genetic effect on survival probability. We also evaluated overall allele bias in the collective sample of young and old workers to assess potential pre-emergence mortality factors.

2.3. Microsatellite selection and worker genotyping

Based on the results of the SNP analyses and our hypothesis that pln and aff QTL would affect life expectancy, 10 genomic regions were studied in the HBC and the LBC for a genetic effect on lifespan using microsatellite markers. Several microsatellite loci were selected from previous studies (Solignac et al., 2007) or identified de novo from the honey bee genome sequence (Honeybee Genome Consortium, 2006) close to each QTL and SNP of interest. The suitability of loci to serve as markers was tested by genotyping eight individuals per cross. One microsatellite per region of interest was selected based on its reliability. The selected microsatellite loci were genotyped in 48 young and 48 old workers of a given backcross.

A tailed-primer approach (Schuelke, 2000) was used, in which the microsatellite alleles were labeled with a universal M13-primer coupled to an IRD700™ or IRD800™ color label (Licor Inc., Lincoln, NB), for detection with a DNA Analyzer 4300™ (Licor Inc., Lincoln, NB). A touchdown PCR protocol was used for the amplification of the microsatellite alleles, decreasing the annealing temperature from 68° to 48° C (Schug et al., 2004). PCR reactions were carried out in 10 μl total volume: 1 μl of 10 ng/μl of template DNA was added to a master mix that contained 1 μl of 2 mM dNTPs, 0.25 μl of 10 μM forward primer, 0.5 μl of 10 μM reverse primer, 0.5 μ of 1 μM of IRD-labeled M13-primer, 1 μl of 10× PCR buffer (20 mM $MgCl_2$, 100 mM Tris-HCl [pH 8.3], 500 mM KCl), 0.13 μl of 5 u/μl Taq DNA polymerase, and 5.62 μl dH_2O. PCR products of different size and IRD label were combined before loading on 25 cm polyacrylamide gels. Samples were run at 1,000 V for 1.5-2.5 h, depending on product size to determine which of the two alternative alleles each individual was carrying. The significance of allele frequency shifts from young to old ages was assessed by Fisher's exact tests of a 2 × 2 contingency table, as described above.

3. Results

The SNP analyses identified five markers in the HBC and five additional markers in the LBC that showed a shift in allele frequencies between the young and old worker cohorts, suggesting a linked genetic effect on survival probability between the ages of 1 and 40 days. The 10 effects were significant without correction for multiple comparisons (Table 1) but only the effect of

ahb687 in the HBC remained significant (Fig. 2) after Bonferroni-correction for all tests performed. None of these loci showed a corresponding effect in the alternate backcross (Table 1). In addition, 11 loci showed an overall deviation from the expected Mendelian 1:1 ratio when combining the young and old samples (Table 2). Six of these loci remained significant after Bonferroni correction. None of the loci showed a corresponding deviation from 1:1 in the alternate backcross (Table 2). Two loci (est6107, est73) showed an overall skew in allele distribution and a shift in allele frequency between young and old.

Table 1. SNP markers that show an allele frequency shift from young to old workers.

Marker	Genomic location: scaffold (bp position in scaffold)	Backcross	Allelic ratio among young workers	Allelic ratio among old workers	Uncorrected *p*-value	*p*-value in alternative backcross
Ahb687	1.7 (618366)	HBC	1/18	16/3	0.0001	0.5118
Ahb1303	1.42 (295472)	HBC	16/4	9/10	0.0484	0.2049
Est6107	8.7 (1097110)	HBC	9/5	19/0	0.0084	0.5148
Est8873	11.18 (430913)	HBC	4/15	10/8	0.0448	Monomorphic
Ahb4126	13.12 (3020860)	HBC	7/13	16/4	0.0095	0.3431
Est73	1.22 (519453)	LBC	0/20	5/14	0.0202	0.1516
Est5253	6.16 (840952)	LBC	13/7	4/15	0.0095	0.5231
Ahb4188	14.15 (509703)	LBC	14/6	6/13	0.0256	0.2049
Est3328	4.13 (1709888)	LBC	6/14	13/6	0.0256	0.7524
Est10281	14.14 (100440)	LBC	13/7	4/15	0.0110	Monomorphic

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Table 2. SNP markers that show a deviation from the expected 1:1 ratio across both age groups.

Marker	Genomic location: scaffold (bp position in scaffold)	Backcross	Allelic ratio among all workers	*p*-value	*p*-value in alternative backcross
Ahb11676	1.41 (394070)	HBC	27/11	0.0094	0.7389

Marker	Genomic location: scaffold (bp position in scaffold)	Backcross	Allelic ratio among all workers	*p*-value	*p*-value in alternative backcross
Ahb6325	3.12 (192748)	HBC	31/9	0.0005	0.4233
Est3280□	4.10 (115698)	HBC	28/1	0.0001	Monomorphic
Est6069 □	8.6 (686197)	HBC	39/1	0.0001	Monomorphic
Est6107	8.7 (1097110)	HBC	28/5	0.0001	1.0
Ahb10360	9.12 (1822605)	HBC	26/13	0.0374	0.7456
Est8456	11.18 (1512912)	HBC	11/24	0.0280	1.0
Ahb3288	12.13 (2062194)	HBC	22/10	0.0339	0.4111
Est10746 □	14.13 (196630)	HBC	1/39	0.0001	Monomorphic
Est73	1.22 (519453)	LBC	5/34	0.0001	0.0630
Est7824	10.28 (209161)	LBC	26/13	0.0374	1.0

□ Result may be due to a single genotyping error in a monomorphic marker.

Polymorphic microsatellite markers near the genomic regions of interest were successfully identified for 10 of the genomic regions in the HBC and seven of the genomic regions in the LBC (Table 3), allowing specific tests of genetic effects. One of these markers (*4A001*), in the *aff3* QTL region was found to have a significant effect in the HBC (Fig. 3) but no other significant effects were identified in either backcross (Table 3). Three of the microsatellite loci (*BI183*, *Ap016*, *HYAL*) showed a significant deviation from an overall 1:1 Mendelian segregation ratio (Table 3), including the microsatellite near the *pln3* QTL in the LBC.

Table 3. Results of specific tests of microsatellites at genomic regions of specific interest.

Marker	Genomic location (Amel4.5)	Backcross	Reason for testing (inter-marker distance in bp)[a]	Allelic ratio among young workers	Allelic ratio among old workers	Uncorrected *p*-value	Overall deviation from 1:1 ratio
Ap106	1.8	HBC	*ahb687* (148,253)	26/18	19/26	0.1395	0.9156
BI159	1.16	HBC	*pln3*	24/20	24/21	1.000	0.4581
K0122	1.37	HBC	*pln2*	22/20	25/16	0.5086	0.2273

Marker	Genomic location (Amel4.5)	Backcross	Reason for testing (inter-marker distance in bp)[a]	Allelic ratio among young workers	Allelic ratio among old workers	Uncorrected *p*-value	Overall deviation from 1:1 ratio
At154	1.42	HBC	*ahb1303* (389,709)	20/16	17/19	0.6376	0.8137
4A001	4.13	HBC	*aff3*	13/20	27/14	0.0345	0.4855
At164	5.12	HBC	*aff4*	9/10	11/11	1.000	0.8759
At168	8.7	HBC	*est6107* (91,081)	19/15	15/21	0.3387	0.8111
K1118	11.18	HBC	*aff2 / est8873*(1,072,084)	25/21	22/22	0.833	0.6733
SV244	13.7	HBC	*pln1*	25/20	21/22	0.6697	0.6698
BI183	13.12	HBC	ahb4126 (70,334)	3/42	6/38	0.3148	0.0001
Ap016	1.15	LBC	*pln3*	17/29	19/26	0.6709	0.0464
K01105B	1.31	LBC	*pln2*	15/18	15/24	0.6341	0.1573
BI152	5.13	LBC	*aff4*	21/23	23/22	0.8331	0.9156
UN112	6.17	LBC	*est5253* (838,765)	28/16	21/20	0.2781	0.1585
BI269	11.18	LBC	*aff2*	22/25	18/29	0.5317	0.1487
SV244	13.7	LBC	*pln1*	23/20	19/19	0.8254	0.7389
HYAL	14.14	LBC	*est10281* (705,999)	29/18	29/18	1.000	0.0233

a SNP markers are given with their distance to the microsatellite but this is not possible for the QTL due to their unknown exact location.

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4. Discussion

Multiple genetic influences on the life expectancy of honey bee workers were detected in this study. These comprised effects of genetic markers near previously mapped QTL. However, more pronounced effects were found elsewhere in the genome, including loci with antagonistic pleiotropy between early and late survival. Results in the two investigated reciprocal backcrosses differed significantly, indicating the importance of genetic background.

Our results suggest that the behavioral QTL aff3 pleiotropically influences adult honey bee worker mortality, in addition to its influence on the age of first foraging (Rueppell, 2009). Our results also suggest a putative influence of pln3 on juvenile survival in addition to its influence on foraging specialization (Hunt et al., 1995), the age of foraging onset (Rueppell et al., 2004b), sucrose responsiveness (Rueppell et al., 2006), and ovary size (Graham et al., 2011). Therefore, the data support our prediction that the QTL, previously identified for influencing the pollen hoarding syndrome, influence worker mortality. This prediction was based on the phenotypic association of worker lifespan with the pollen hoarding syndrome, particularly the age of first foraging (Amdam et al., 2007 and Rueppell et al., 2007). As a consequence of this association, alternative alleles that influence the age of first foraging should influence worker lifespan. However, we also identified several putative genetic influences on worker life expectancy in other regions of the genome, and not every investigated QTL marker influenced worker life expectancy.

Tests of previously identified QTL for influences on worker life expectancy depend on the segregating genetic variation in a particular study population, the genetic background, parental imprinting effects, and the availability of closely linked genetic markers. Therefore, we would not expect to identify all predicted effects, even with large sample sizes. We investigated a marker for 11 of the total 14 possible QTL tests, identifying a significant shift in allelic frequency between newborn and aged workers at the marker locus for aff3 (Fig. 3). This suggests that aff3 influences the life expectancy of adult worker honey bees but it does not rule out influences of all other QTL. Specifically, we also found an influence of the SNP est8873 genotype on survival probability. This SNP is linked to aff2 (also referred to as affnew; Rueppell, 2009), which indicates an influence of aff2 on worker mortality, although the corresponding microsatellite marker did not show a significant effect.

The SNP analysis covered only a part of the honey bee genome and the number of investigated markers was insufficient for a comprehensive QTL mapping analysis. Nevertheless, the SNP screening revealed several effects despite the small sample size. The allele frequencies at the SNP locus ahb687 differed between the young and the old worker groups. The allele that was much more common than its counterpart in the young workers was only found in a small number of the old individuals. This shift in allele frequencies with age cannot be explained by a technical genotyping error, particularly in light of the non-significant results at this locus in the reciprocal backcross. Thus, ahb687 marks a genomic region that exhibits antagonistic pleiotropy. One allele conferred a survival advantage during the developmental phase until adult emergence, leading to an overrepresentation of the “B” allele (95%) in newborn bees. The opposite allele was associated with a survival advantage during adulthood, explaining the overrepresentation of the “A” allele (84%) in 40-day old bees. While the skew in the young bees might also be due to a

distortion of the Mendelian 1:1 ratio before fertilization (Hurst and Werren, 2001), the reversal of allelic skew in the old cohort can only be explained by allele “A” conferring a strong survival advantage in adults.

The genomic region around this locus contains multiple genes that might be interpreted as positional candidate genes for influencing survivorship. The adipokinetic hormone receptor (also known as insect gonadotropin-releasing hormone receptor) gene is the most notable, because it influences nutrient metabolism and starvation resistance in Drosophila (Gronke et al., 2007) and has been linked to aging in vertebrates (Wang et al., 2010). However, our experiment did not identify any specific causes of death. Therefore, the identified genetic effects could be due to a change in the internal rate of aging, altered extrinsic mortality risk, or an interaction between genetic and environmental factors. In the case of the aff QTL, the genetic factor accelerates the intrinsic rate of maturation (Rueppell et al., 2004b), exposing workers earlier to a more hazardous environment (Rueppell et al., 2007). None of the other nine SNP results remain significant after stringent Bonferroni correction. Nevertheless, these SNPs represent candidate regions for further investigation. The SNP est6107 is particularly interesting because its allelic ratio in the young workers was close to the expected 1:1 ratio, but only one allele was found in the old worker group of the HBC.

Est6107 was also among the six loci that showed a significant skew in their allele distribution when both age groups were combined, indicating an influence on combined juvenile and adult survivorship. Three of these loci (est3280, est6069, est10746) contained only one individual with the less common genotype and are monomorphic in the reciprocal backcross. Thus, it is likely that these three loci are spuriously categorized as polymorphic and the results were due to genotyping errors. In contrast, the results of est6107 and the remaining two loci ahb6325 and est73 cannot be attributed to genotyping errors that would favor one allele over the other because these loci showed no significant skew in the reciprocal backcross (Table 2). Instead, it can be concluded that the underrepresented alleles at these three loci led to pre-emergence mortality or that the overrepresented alleles influenced meiotic segregation in their favor (Hurst and Werren, 2001). The existence of lethal alleles in honey bees would be remarkable because haplodiploidy strongly selects against them (Hedrick and Parker, 1997).

All identified effects were backcross specific and thus influenced by genetic background, similar to the QTL effects in all our previous studies (Graham et al., 2011, Rueppell et al., 2004b, Rueppell et al., 2006 and Rüppell et al., 2004). We cannot exclude the possibility of allelic scoring bias for the microsatellites because these loci were typically genotyped only in one backcross. Thus, we do not further consider the microsatellite loci that show an overall allelic

bias. It is impossible to point to candidate genes for the juvenile mortality effects because lethal genes may belong to a variety of groups (Spradling et al., 1999) and the genome environment of the three SNP markers of significant overall bias are extremely gene rich, with over one gene per 2000 base pairs on scaffold 1.22, on scaffold 3.12, and in the 400000 base pairs surrounding est6107 on scaffold 8.7.

We sought to confirm the identified SNP effects in a larger sample with microsatellites that were linked to the SNPs with an effect on adult survival. However, this confirmation of the SNP results was unsuccessful. Most likely, this is due to the distance between the SNP and corresponding microsatellite markers (70000–100000 base pairs). Physical genome distances translate into substantial recombination distances between our SNP and microsatellite markers due to the very high recombination rate throughout the honey bee genome (Beye et al., 2006). However, we also cannot exclude the possibility that errors in our microsatellite genotyping eroded the marker effect. In contrast, Sequenom's SNP genotyping has a very low reported error rate (Kathiresan et al., 2008), suggesting that the initial SNP results are trustworthy. However, follow-up studies are needed to reconfirm the effects and test how general they are.

More genetic effects of the aff and pln QTL might have been expected because the age of first foraging strongly influences longevity in honey bee workers (Rueppell et al., 2007 and Rueppell et al., 2008). The pollen hoarding syndrome affects worker lifespan physiologically by influencing the dynamics of juvenile hormone and vitellogenin (Nelson et al., 2007) and behaviorally by influencing foraging initiation and foraging specialization that in turn affect worker lifespan (Rueppell et al., 2007). However, QTL effects are always dependent on the segregating variation in the particular cross. The high and low pollen hoarding strains are not comparable to inbred lines (Page and Fondrk, 1995) and this experiment was conducted multiple generation after the isolation of the aff and pln QTL with at least one outbreeding event. In addition, the exact locations of the QTL are unknown and any marker is only linked to the actually effective genetic element to a certain degree, which makes the detection of weak effects difficult. Furthermore, the QTL may display antagonistic pleiotropy, shortening the pre-foraging lifespan but extending foraging lifespan. Therefore, the QTL tests are conservative and previous studies have shown that usually only a fraction of the tested QTL show an effect in any particular cross (Graham et al., 2011, Rueppell et al., 2006, Rüppell et al., 2004 and Wang et al., 2009).

In this study, we have identified natural genetic effects on honey bee worker lifespan for the first time. Some of these effects were quite strong, causing significant shifts in the allelic distributions from newly emerged to 40-day-old honey bees. The strongest effect shifted the frequency of a particular allele from 5% in young bees to 84% in old bees, which amounts to a 16-fold

difference. The size of the effect contradicts the suggestion that genetic effects may only account for up to 35% of the observed lifespan variability in invertebrates (Finch and Tanzi, 1997). Although our data is not suitable to calculate how much lifespan variability is explained by our loci, the magnitude of the identified effects is similar to the pronounced effects of several experimental mutations that have been studied in invertebrates (Kenyon et al., 1993 and Paaby and Schmidt, 2009).

Furthermore, we found clear evidence for antagonistic pleiotropy, one of the major evolutionary explanations for the evolution of aging (Williams, 1957). While the honey bee displays an exceptional plasticity of aging (Omholt and Amdam, 2004) and is lacking a list of well-characterized “aging genes” that exist in the classic aging models, our study suggests that fundamentally similar genetic patterns and processes exist, even in species with a highly derived life history (Rueppell et al., 2004a and Rueppell et al., 2007).

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