Major Quantitative Trait Loci Affecting Honey Bee Foraging Behavior

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ABSTRACT

We identified two genomic regions that affect the amount of pollen stored in honey bee colonies and influence whether foragers will collect pollen or nectar. We selected for the amount of pollen stored in combs of honey bee colonies, a colony-level trait, and then used random amplified polymorphic DNA (RAPD) markers and interval mapping procedures with data from backcross colonies to identify two quantitative trait loci (phn1 and phn2, LOD 3.1 and 2.3, respectively). Quantitative trait loci effects were confirmed in a separate cross by demonstrating the cosegregation of marker alleles with the foraging behavior of individual workers. Both phn1 and phn2 had an effect on the amount of pollen carried by foragers returning to the colony, as inferred by the association between linked RAPD marker alleles, D8-Sf and 301-55, and the individual pollen load weights of returning foragers. The alleles of the two marker loci were nonrandomly distributed with respect to foraging task. The two loci appeared to have different effects on foraging behavior. Individuals with alternative alleles for the marker linked to phn2 (but not phn1) differed with respect to the nectar sugar concentration of their nectar loads.

The number and effects of genes influencing naturally occurring behavioral traits is an issue of great importance to studies of behavior and behavioral evolution. Current evolutionary theory assumes that most adaptive traits are polygenic in inheritance, influenced by many genes, each having infinitesimally small additive effects (Barton and Turelli 1989; Orr and Coyne 1992; Lai et al. 1994). This assumption of polygenic inheritance is particularly strong in quantitative and behavioral genetics. However, there have been few studies that were sufficient to determine the numbers and relative effects of genes on adaptive traits. These determinations can be made when mapping quantitative trait loci (QTL) involved in particular crosses. Most QTL mapping studies of crop plants have revealed a few major loci with relatively large effects and additional loci with smaller effects (reviewed by Tanksley 1993).

Single genes or loci with major effects have been identified for behavioral disorders of humans, mice, rats, and Drosophila (Gilliam 1992; Cardon et al. 1994; Crabbe et al. 1994; Hall 1994; McKlusick 1994; Takahashi et al. 1994). For example, QTL with major effects on behavior have been found for human behavioral traits such as dyslexia (Cardon et al. 1994) and Alzheimer’s disease (St. George-Hyslop et al. 1990). Behavioral genes have also been mapped in studies of Drosophila mutants (reviewed by Kriaucou and Hall 1994). There are only a few mapped loci affecting behavioral traits in nonlaboratory animal populations. Examples include a sex-linked locus for mice with effects on aggression (Carlier et al. 1990; van Oortmerssen and Sluyter 1994) and recently, the dominant locus, for, which controls a component of foraging behavior in Drosophila larvae (de Belle et al. 1989, 1993). Mapping studies to find loci that affect animal behavior are impeded in many of the commonly studied species by large environmental effects and by the difficulties of testing large progeny sets.

The honey bee, Apis mellifera, offers advantages as an experimental organism for studying the behavioral genetics of foraging. Honey bees are social insects that live in large colonies, so that many individuals can be generated and tested at one time. The single reproductive female, the queen, mates with ~12–17 haploid males (reviewed by Page 1986) to produce as many subfamilies as possible in the colony, but artificial insemination may be used to control matings (Laidlaw 1977). Nonreproductive females (workers) from the same subfamily share an average of 75% of their genes by descent, because a haploid male (drone) transmits an identical genome to each of his worker progeny. Therefore, in colonies containing a queen mated to a single drone, the genetic similarity of workers within each colony makes it easier to assess the paternal contribution to worker behavior when comparing between colonies. Behavioral variation among subfamilies has been found for pollen foraging (Calderone and Page 1988, 1991; Page and Robinson 1989, 1991; Oldroyd et al. 1991), foraging distance (Oldroyd et al. 1993) and floral resource preference (Oldroyd et al. 1992), thus demonstrating heritable variation for these traits. Interactions between subfamilies (genotypes) and colony environments have also been observed (Calderone and Page 1992).
The pollen-hoarding trait (storing pollen in the nest) of the honey bee has relatively high heritability and can be selected as a colony-level phenotype (Helmich et al. 1985). Recently, high and low pollen-hoarding strains of honey bees were established by selecting for the amount of pollen stored in the combs of the nest (Page and Fondrk 1995). The effect of the colony-level selection was to differentially change the proportion of pollen foragers in the high and low strain colonies. The effect of two-way selection was observable at the level of amounts of stored pollen within the colony, as well as at the level of individual foraging behavior. Therefore, we crossed high and low pollen-hoarding lines to produce a mapping population of colonies for detecting QTL involved in pollen-hoarding behavior and to study the effects of the QTL on individual behavior. Here we report the map location of two such loci and their effects on both colony and individual phenotypes.

**MATERIALS AND METHODS**

**Detection of pollen-hoarding QTL at the colony level:** We conducted five generations of two-way selection to establish strains of bees that hoarded high and low quantities of pollen based on the amount of pollen stored in the combs of the hive (Page and Fondrk 1995). After two generations, a low-strain virgin queen was instrumentally inseminated with semen of a male derived from a generation 2 queen of the high strain (Laidlaw 1977). An F1 virgin queen was produced and exposed to CO2 to induce egg laying, without mating; normal males are genetically haploid and derived directly from the unfertilized gametes of queens. A generation 2 high-strain virgin queen was instrumentally inseminated with the sperm of a male derived from her mother to produce inbred, high-strain virgin queens (see Figure 1A). Males derived from unfertilized eggs of one F1 queen were mated individually to inbred high-strain virgin queens. This backcross was made to the high line because of evidence that high pollen-hoarding behavior is a recessive trait (Page et al. 1995). Colonies consisting of backcross worker progeny were established from these queens. Thirty-eight of these colonies were evaluated for the amount of stored pollen by measuring the area of wax cells filled with pollen in all of the combs in each colony (Page and Fondrk 1995). Measurements were made in an almond orchard near Davis, California during the spring bloom in the first week of March, 1992.

**Linkage analyses of RAPD markers for colony-level data:** DNA was extracted from 95 haploid male progeny of the F1 queen and was used to construct a saturated genomic map with RAPD markers (Hunt and Page 1995). RAPD markers (Williams et al. 1990) were produced by amplifying honey bee DNA in PCR using commercially available 10-base oligonucleotides as primers, as previously described (Hunt and Page 1995). Markers were resolved in gels containing 1–1.2% Synergel (Diversified Biotech, Newton Centre, MA), 0.7% agarose and 0.5X TBE. The QTL analyses compared the in-

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**FIGURE 1.**—Backcross schemes for studying pollen-foraging quantitative trait loci. (A) Crossing scheme for detection and mapping of colony-level QTL for pollen-foraging (measured as area of pollen stored in combs) was based on the contribution of QTL from segregating haploid drone fathers of each colony. The cross to produce the F1 queen was made after the second generation of selection. Segregating haploid drones were each individually mated to a single virgin queen. The source of unfertilized eggs resulting in haploid drones is shown by dashed arrows. (B) Crossing scheme to confirm effects of QTL on individual foraging behavior based on segregation in worker progeny of the F1 queen in a single colony. The cross to produce the F1 queen was made after the fifth generation of selection.

Pollen and nectar foraging in the honey bee are complex behaviors expressed in a social context. Pollen is an important resource for honey bee colonies because it serves as the protein source for raising brood. The quantity of pollen stored in the comb is regulated by both positive and negative stimuli, such as the presence of brood and the size of the pollen stores (Free 1967; Free and Williams 1971; Al-Tikrity et al. 1972; Cama\-zine 1993). As new sources of pollen and nectar become available, foragers are recruited to specific resources through a system involving recruitment dances (Von Frisch 1967; Seeley 1985; Michelsen et al. 1992). Individual bees respond to removal of colony pollen stores with both increased foraging effort and the recruitment of new foragers to pollen resources until pollen stores return to previous levels (Fewell and Winston 1992). In addition to environmental stimuli, the probability that an individual worker will forage for pollen vs. nectar also depends on her genotype.
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FIGURE 2.—Major linkage groups containing the most likely locations of QTL plnl and pln2 as determined by Mapmaker and Mapmaker-QTL (LANDER et al. 1987; LANDER and BOTSTEIN 1989). Linkage group designations (II and X) are from HUNT and PAGE (1995). Numbers to the left of the bar are distances in cM between markers. Numbers to the right designate specific RAPD markers. Numbers preceded by an alphabetic character were produced by Operon Technologies, Alameda, California, while those without an alphabetic character were produced by University of British Columbia Biotechnology Laboratory. Numbers following the dash represent the size of the RAPD marker fragment in kb. For instance, linkage group II, marker D8-3f, is a fragment amplified with Operon primer D8 and is 0.3 kb in length. A final letter designation of "f" shows that this particular marker is a fragment length polymorphism while those followed by "h" are fragment length polymorphisms that form distinguishable heteroduplexes (HUNT and PAGE 1992). The line tracing to the right shows the LOD score for the likelihood that a QTL exists at each location along the linkage group.

heritance of specific RAPD markers from each drone with pollen stores within the colony that the drone sired. Interval mapping with Mapmaker-QTL software (LANDER and BOTSTEIN 1989) was used to screen 364 markers belonging to 26 linkage groups that each contained at least three RAPD markers, spanning 3100 cM of the honey bee genome at an average spacing of ~9 cM (HUNT and PAGE 1995).

Confirmation of QTL effects with individual behavior: A second F1 cross was made between two individuals from the fifth generation of selection for pollen-hoarding behavior (Figure 1B). An F1 queen was mated to a single drone from a high pollen line. Three thousand newly emerged adult workers from the resulting colony were marked with paint. Marked workers were collected as they returned from foraging trips carrying nectar and/or pollen over a 3-day period during warm weather of August, 1993 (near Davis, CA). Returning foragers were collected, and nearly all of the marked individuals that were currently engaged in foraging were removed by the end of the 3-day period. The bees were anesthetized with CO2 within 10 min of collection, and the weights of individual pollen and nectar loads were recorded. The nectar was withdrawn from each forager by squeezing the bee so that the nectar entered a capillary tube. The relative sugar concentration of the nectar load was determined with a hand-held refractometer (GARY and LORENZEN 1976; ROBINSON and PAGE 1989). Pollen was removed from the corbiculae on legs of individual pollen foragers and weighed. The few individuals that were not carrying pollen or nectar were discarded. Three hundred and thirty-two foragers were frozen on dry ice, and DNA was extracted from each individual (HUNT and PAGE 1995). The inheritance of RAPD markers linked to the two putative pollen-foraging QTL was then correlated with individual foraging behavior. We used data from one informative RAPD marker locus linked to each of the two QTL to infer the QTL genotype. Some error in scoring QTL genotypes is expected because we did not have informative flanking markers. RAPD markers from individual lanes that were considered too difficult to score were entered as missing data.

Statistical methods: Bees often specialize on either pollen or nectar, resulting in relatively large classes of individuals with zero values for load weights for each resource and hence a bimodal distribution for both pollen and nectar load weights. The assumptions of analysis of variance (ANOVA) were met when only the individuals with nonzero values were used in analyses, so individuals that did not carry pollen or nectar were eliminated from those analyses. Analysis of variance was used for data sets with nonzero values for the three variables pollen load weight, nectar load weight and nectar concentration. In addition, nonparametric methods were used on complete data (with zero values) to correlate individual foraging behavior with the inheritance of RAPD marker alleles at the two loci. Weights of pollen and nectar loads and nectar concentrations were first compared using a Kruskal-Wallis test for individuals with alternative alleles at the two loci (SOKAL and ROHLF 1981). When a statistically significant Kruskal-Wallis test was obtained, Mann-Whitney U-tests were applied to data from individuals with alternative maternally inherited alleles at each locus. The effects of the two pollen-collecting QTL were also evaluated by separating individual foragers into task groups (collecting pollen only,
nector only, or both) and performing a G-test for heterogeneity (SOKAL and ROHLF 1981). This analysis emphasized foraging choice, independent of load size. Finally, the correlation of nectar concentration and nectar load sizes carried by returning foragers was evaluated by linear regression for alternative alleles and for task groups.

RESULTS

Colony-level QTL mapping: The 38 haploid drone progeny of the first F1 queen were analyzed for RAPD markers after they were individually backcrossed to sister queens from the high-pollen line (Figure 1A). As a consequence of male haploidy, backcross workers within each of the 38 colonies inherited the same genome from the drone father of that particular colony. The high-strain queen mothers of the colonies each shared an average of ≥87.5% of their genes by common descent because of male haploidy and inbreeding. QTL analyses for the total area of pollen stored in colonies showed that one marker interval, D8-3f to 388-76f (Figure 2A), had a LOD score (logarithm of odds ratio) for likelihood of containing a QTL of 3.1 and explained 38% of the total phenotypic variance. This putative QTL was designated pln1. Another interval, P13-67f to S15-16 (pln2), contained a putative QTL with a LOD of 2.3 that explained 33% of the total phenotypic variance (Figure 2B). In combination, the two loci explained 59% (LOD 5.3) of the phenotypic variance. Flanking marker data showed that the high-pollen pln1 allele was inherited by the F1 queen from her low-strain parent, the high pln2 allele had a high-strain origin. The low-strain origin of pln1 can be explained by lack of fixation for all QTL after just two generations of selection or by chance fixation of some QTLs among strains as a consequence of the initial establishment of the strains. It is also possible that this QTL has an overdominant effect, in which case it would appear that a high allele had been inherited from the low strain in the backcross.

Confirmation of the effects of pln1 and pln2 on individual behavior: High-strain foragers individually are more likely to return from foraging trips with loads of pollen than are workers of low-pollen hoarding strains (CALDERONE and PAGE 1988, 1991; PAGE et al. 1995). Therefore, to confirm the behavioral effect of the QTL, we backcrossed a second F1 queen from generation 5 to a generation 5 high-strain drone (Figure 1B). In this cross workers differed for both putative QTL and their linked RAPD markers only as a consequence of meiotic recombination in the F1 queen. Data for pollen and nectar load sizes and nectar concentration of incoming foragers were not normally distributed because there were many zero values for each variable (Figure 3, A and B). In addition, nectar and pollen loads of incoming foragers were not independent variables because they were inversely correlated ($r^2 = 0.513$) (Figure 3C). This correlation was significant, even if we considered only those individuals that carried both pollen and nectar ($r^2 = 0.174$, $p < 0.0001$).

We determined the inheritance of alleles from two RAPD marker loci linked to pln1 and pln2 (D8-3f and 301-55, respectively). RAPD-marker alleles at D8-3f and 301-55 did not have a detectable effect on viability or on the likelihood of becoming a forager, because the alleles at both loci segregated according to the expected 1:1 ratio ($p = 0.845$ and 0.506; $G = 0.038$ and 0.443, respectively, 1 d.f.). The alleles at the two marker
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![Figure 4](https://example.com/figure4.png)

**Figure 4.**—Proportion of honey bee foragers of each genotype performing different tasks. (A) The genotypic categories show the maternal contribution of RAPD marker alleles linked to pollen-hoarding QTL. *phn1* and *phn2* (RAPD) markers D8-3f and 301-55, respectively. Individual returning foragers were separated into three task groups based on whether they were carrying only pollen (Pollen), both pollen and nectar (Both) or just nectar (Nectar). Individuals were further separated according to which RAPD marker allele linked to pollen foraging QTL that they inherited from the F1 queen. RAPD marker alleles are used to infer QTL alleles, but some error in assigning individuals to specific QTL genotypes is expected due to recombination between the single RAPD marker linked to each QTL. The designations HS and LS refer to the high-strain and low-strain origins of the RAPD marker alleles. For the QTL, *phn1* linked to D8-3f, the high-pollen allele was inherited from the low-strain parent in both sets of crosses used in this study. Workers were nonrandomly distributed between genotypic and task group categories (G = 16.48, p = 0.011, n = 320, 6 d.f.) and also were biased by task group at each of the two marker loci, D8-3f and 301-55 (G = 8.140 and 6.486, p = 0.017 and 0.039, n = 325 and 327, respectively, 2 d.f.). The bias was in the direction predicted by colony-level data. (B) The same task groups were compared when individuals were grouped into the three possible QTL genotypes at both loci that resulted from this backcross. For ease of presentation, the inferred QTL genotype is shown rather than the strain of origin of marker alleles. The “High” allele of *phn1* actually had a low strain origin (*phn1-LS = High*). There was significant heterogeneity between genotypes and foraging tasks (G = 15.5, p = 0.004, n = 320, 4 d.f.).

Loci also were inherited in workers independently, because there was no significant skew from 1:1:1:1 (p > 0.05, G = 3.583, n = 320, 3 d.f.). The four RAPD-marker genotypes varied significantly for weight and concentration of nectar and for pollen load weight (p = 0.014, 0.010 and 0.009, respectively, Kruskal-Wallis test). Genotypes also varied with respect to the proportion of the total load weight (nectar plus pollen) that was pollen (p = 0.007, Kruskal-Wallis test). This may be the more appropriate test because of the lack of independence of the two variables.

Effects on load weights were also observed at each of the two marker loci. Inheritance of alternative alleles for marker D8-3f was significantly associated with the weight of pollen and nectar carried (p = 0.013 and 0.026, respectively, n = 325, Mann-Whitney U-test). As in the case of the colony-level analyses, the high-pollen QTL allele linked to D8-3f that resulted in larger pollen loads (and smaller nectar loads) was inherited from the low-strain parent. Inheritance of marker alleles of 301-55 also was associated with pollen and nectar load weights (p = 0.012 and p = 0.029, respectively, n = 327, Mann-Whitney U-test). Allelic substitutions at the two marker loci, D8-3f and 301-55, explained 1.7 and 1.1%, respectively, of the total variance for pollen load weights of individual foragers in this backcross. When analyzed in combination, they explained 3.5% of this variance. This contrasts with the colony-level analyses, in which allelic substitutions at these two marker loci explained 9.5 and 18.3% of the colony-level phenotype variance for the quantity of pollen stored (and 29.8% when analyzed in combination). Inheritance of RAPD marker alleles of D8-3f and 301-55 did not have significant effects on nectar or pollen load weights when the zero values were excluded from analyses.

The correlation of marker alleles, linked to *phn1* and *phn2*, with individual forager behavior can be demonstrated by separating returning foragers into task groups that reflect foraging choices: those that carried only pollen loads, those that had both pollen and nectar and those that had only nectar (n = 48, 122 and 162, respectively). Each of these three groups can then be separated into their QTL genotypes, as inferred from the linked markers. Since all of the workers inherited the same high-strain (HS) marker allele from the haploid drone father, workers were homzygous at a marker locus if they also inherited the HS allele from the F1 queen and were heterozygous at the marker locus if they inherited the maternal low-strain (LS) allele. The maternal marker-allele contribution to worker progeny in the three foraging task groups is shown in Figure 4A. Overall, the data showed a nonrandom distribution of workers between RAPD marker genotypes and task-group categories (G = 16.481, p = 0.003, n = 320, 6 d.f.) (Sokal and Rohlf 1981) and, the distribution among task groups also was biased at each of the two marker loci, D8-3f and 301-55 (G = 8.140 and 6.486,
$p = 0.017$ and $0.039$, $n = 325$ and 327, respectively, 2 d.f.). These results demonstrate a bias toward specific tasks in the direction predicted by the colony-level data.

No difference in task group could be detected between individuals that were heterozygous only at D8-3f compared to those that were heterozygous only at 301-55, suggesting additivity across the two loci. We therefore pooled the latter two groups and tested for heterogeneity of the two-locus genotypes: those that inherited two maternal "high-pollen" QTL alleles, those that inherited only one, and those that inherited no high-pollen alleles from the F1 queen (as inferred by marker
Inheritance of alternative marker alleles for 301-.55 was associated with the nectar concentration in the crops of returning foragers that had nectar ($p = 0.0017$, $n = 302$, one-way ANOVA). The high-strain RAPD marker allele was associated with lower sugar concentration. In contrast, inheritance of alleles of marker D8-.3f had no significant effect on nectar concentration ($p = 0.672$, $n = 301$). Another way to analyze the effect of pln2 on concentration is to look at the relationship between nectar concentration and nectar load size of returning foragers. The regression of nectar concentration on nectar load size has a significantly positive slope for the maternal high-strain QTL allele of pln2 (as inferred from the linked marker), there was no significant relationship between load size and concentration. Similarly, individual nectar foragers that also carried pollen showed a lack of correlation between nectar load size and concentration (Figure 5C). The correlation coefficients for individuals carrying the alternative alleles of 301-.55 (linked to pln2) were significantly different ($p < 0.05$). This relationship demonstrates a differential response to sugar concentration between individual foragers with alternative alleles for pln2, but not for plnl.

**DISCUSSION**

We have demonstrated that two loci have major effects on the amount of stored pollen in colonies and detectable effects on individual foraging behavior. These two QTL explained roughly 59% of the total phenotypic variance for quantities of stored pollen in our backcross population of colonies. Although the estimates of the individual effects of these two QTL on colony pollen stores are in the upper range reported for any QTL from mapping studies (Tanksley 1993), our small sample size of 38 colonies greatly reduces the precision for estimating QTL effects and may be biased upward (Beavis 1994). In spite of the uncertainty concerning the magnitude of QTL effects, two lines of evidence indicate that both of these loci must have a major influence on pollen foraging behavior. The first evidence that these loci have a major effect is that we were able to identify them with only 38 colonies. Simulations have shown that backcross progeny sets with 100 individuals have little power to detect a single QTL explaining 5% of the phenotypic variance (frequency of correct identification = 0.11) (Van Ooijen 1992). A similar result was obtained with 10 QTLs that each explained 6.3% of the variance in a simulated data set of 100 F2 progeny (frequency = 0.33) (Beavis 1994). The second indication that plnl and pln2 have major effects is the fact that we were able to validate their behavioral effects in a separate cross and using a different phenotype than the one used to map these loci.

The effect of the two QTL was to alter the probability that an individual would collect pollen vs. nectar and, consequently, influenced the quantity of pollen collected and stored by the colony. For one of the two loci identified with effects on foraging behavior (plnl), the high-pollen allele was inherited from the low-pollen strain. QTL with allelic effects that are opposite to what would be expected based on parental strain phenotype are not uncommon (Paterson et al. 1988; Doebley and Stec 1991; De Vicente and Tanksley 1993). It is possible that alleles of plnl are overdominant in expression (the heterozygote having a more extreme phenotype than either homozygote), thus giving the appearance that the low strain had a high QTL allele because we backcrossed to the high-strain parent. We cannot distinguish between overdominance or complementary QTL fixation at this locus because we used backcrosses to one line only. However, other observations suggest an overdominant expression pattern. The quantity of stored honey (processed nectar) in colonies and the nectar collecting preferences of individual foragers both demonstrate overdominant (or heterotic) patterns of inheritance in our selected strains (Page et al. 1995; Page and Fonderk 1995). Further research is needed to determine the degree of dominance or overdominance for plnl and pln2.

Results from this study indicate that plnl and pln2 have different effects on individual forager behavior. Only RAPD marker alleles linked to pln2 had a significant correlation with the relative sugar concentration of the nectar. Figure 5 shows that the correlation of these marker alleles with nectar sugar concentration was due to the relatively low and constant concentrations carried by individuals with the RAPD marker linked to the high-pollen allele of pln2. The lack of a relationship between nectar concentration and load weights for individuals with the high-strain marker linked to pln2 is similar to that observed for pollen foragers that were carrying nectar, but contrasts with the significant relationship observed for individuals with either of the marker alleles linked to plnl. This study is inadequate to determine the specific manner in which these two loci are affecting behavior. However, the availability of markers that are linked to major genes affecting honey bee foraging behavior will enable studies that identify specific genetic components of the behavior and suggest mechanisms of gene action.

The traditional quantitative genetic models of evolu-
tion assume that many genes with small additive effects are responsible for variation in adaptive behavioral traits (Fisher 1958; Barton and Turelli 1989; Orr and Coyne 1992; Lai et al. 1994). However, our data suggest that two loci have a major effect on colony pollen stores and also influence individual foraging behavior. Individual foragers that inherited both RAPD marker alleles linked to the high-pollen QTL alleles were 1.7 times more likely to bring back pollen to the colony than to carry only nectar. Tightly linked markers that flank these two QTL could be used in studies to determine the specific individual effects of these two loci on worker foraging behavior and their contributions to observed phenotypic variability within different honey bee populations.

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