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Aedes aegypti: A Quantitative Trait Locus (QTL) Influencing Filarial Worm Intensity Is Linked to QTL for Susceptibility to Other Mosquito-Borne Pathogens

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BEERNTSEN, B. T., SEVERSON, D. W., KLINKHAMMER, J. A., KASSNER, V. A., AND CHRISTENSEN, B. M. 1995. *Aedes aegypti:* A quantitative trait locus (QTL) influencing filarial worm intensity is linked to QTL for susceptibility to other mosquito-borne pathogens. *Experimental Parasitology* **81,** 355–362. Because intensity of infection was significantly increased in a substrain of *Aedes aegypti* selected for susceptibility to the filarial worm, *Brugia malayi,* experiments were designed to assess numbers of microfilariae (mf) ingested and midgut penetration by mf in this susceptible substrain as compared to a refractory substrain selected from the same parental stock. Refractory mosquitoes ingested significantly fewer mf than susceptible mosquitoes and significantly fewer numbers of mf penetrated through refractory midguts as compared to susceptible midguts. In 16.7% of the refractory midguts, no mf were able to penetrate the midgut and in three refractory mosquitoes over 250 mf were ingested, but no mf penetrated the midgut. These results indicate that permissiveness of the midgut for penetration by microfilariae can determine not only parasite intensity, but also prevalence of infection. The genetic basis for ingestion of mf and midgut penetration was assessed using restriction fragment length polymorphism markers and quantitative trait loci (QTL) mapping. This mapping identified a QTL on chromosome 2, idb[2.LF181] (idb, intensity determinant for *Brugia*), that seems to influence ingestion ability. This QTL is linked to a previously identified QTL for susceptibility to *B. malayi,* fsb[2,LF98], as well as to loci for susceptibility to the malaria parasite, *Plasmodium gallinaceum,* and yellow fever virus. These results suggest that this region of chromosome 2 contains one or more genes that influence susceptibility of *A. aegypti* to several mosquito-transmitted pathogens.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Aedes aegypti;* Diptera; Culicidae; Mosquito; *Brugia malayi;* Nematoda; Filarioidea; Filarial Worm; centimorgan (cM); Filarial worm intensity; Filarial worm susceptibility to *B. malayi* (fsb); Genetic linkage; Intensity determinant for *B. malayi* (idb); Liverpool (LVP); Liverpool female (LF); Logarithm of odds (lod); Microfilariae (mf); Midgut penetration; Phosphoglucomutase (PGM); *Plasmodium gallinaceum* susceptibility (pgs); Quantitative trait loci (QTL); Restriction fragment length polymorphism (RFLP); Vector competency.

INTRODUCTION

Lymphatic filariasis, caused by the filarioid nematodes, *Brugia malayi, Brugia timori,* and *Wuchereria bancrofti,* is a mosquito-borne disease that infects approximately 120 million people in the tropics and subtropics (WHO, 1994). Thirty years ago, classic studies by Macdonald (1962a,b, 1963) and co-workers (Macdonald and Ramachandran 1965; Macdonald and Sheppard 1965) demonstrated that inheritance of susceptibility of the mosquito, *Aedes aegypti,* to *B. malayi* infection was controlled primarily by a sex-linked recessive gene, designated f^m, located on chromosome 1. These studies also suggested that other modifier genes were involved because f^m did not entirely control susceptibility. Additional experiments showed that f^m is the primary gene controlling susceptibility of *A. aegypti,* not only to *B. malayi,* but also to other filarial worms inhabiting the thoracic musculature (Macdonald and Ramachandran 1965).

Although the biochemical effect of f^m is unknown, it has been suggested that its effect occurs within the host tissue lodging the parasite

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and does not directly affect the parasite itself (McGreevy *et al.* 1974). However, parabiotic twinning studies, using *B. malayi* and susceptible and refractory *A. aegypti,* suggested that hemolymph factors present in refractory mosquitoes may prevent parasite development in this strain (Kobayashi *et al.* 1986).

Recently, polypeptide synthesis was assessed in thoraces of *A. aegypti* strains, refractory and susceptible to *B. malayi* infection (Wattam and Christensen 1992a,b). Using *in vivo* labeling followed by two-dimensional gel electrophoresis, six polypeptide differences were observed only in the refractory strains after a bloodmeal. Furthermore, additional work suggested that factors inhibiting *B. malayi* development in the refractory strains were in the thorax, because no polypeptide differences were seen in the hemolymph between the two strains (Wattam and Christensen 1992a).

Efforts are underway to isolate the genes that control susceptibility of *A. aegypti* to *B. malayi,* and recent studies in our laboratory (Severson *et al.* 1994) used a RFLP-based genetic linkage map for *A. aegypti* (see Severson *et al.* 1993) to identify two QTL influencing *B. malayi* susceptibility. The first locus (fsb[1,LF178]) resides on chromosome 1 and exhibits a recessive effect with respect to susceptibility. Severson *et al.* (1994) concluded that this QTL contains the previously described f^m locus because its effect is consistent for susceptibility being recessive and it also maps to the appropriate genomic location. The second locus, fsb[2,LF98], resides on chromosome 2 and exhibits an additive effect on susceptibility. Furthermore, these data suggest that the f^m locus might function to provide a permissive state for *B. malayi* development, but subsequent expression of the susceptible phenotype depends upon the genotype at the QTL on chromosome 2 (Severson *et al.* 1994).

Consequently, since susceptibility to filarial worm infection in *A. aegypti* is a multigenic trait, substrains of *A. aegypti* Liverpool (LVP), highly susceptible (LVP^{sbm}) and refractory (LVP^{rbm}) to *B. malayi* infection, were selected from the susceptible LVP strain by pairwise mating in order to fix susceptibility and refractory alleles in the two populations. Because the newly selected susceptible substrain showed a much higher mean intensity of infection, and because the ability of mf to migrate out of the bloodmeal and penetrate the midgut epithelium is a critical step in the lifecycle of *B. malayi,* we assessed the number of parasites ingested as well as the ability of *B. malayi* to penetrate susceptible as compared to refractory midguts *in vitro.* We then used RFLP markers and QTL analysis to identify the location of gene(s) influencing filarial worm intensity in *A. aegypti.*

Materials and Methods

Mosquito selection for susceptibility to B. malayi. Substrains of *A. aegypti* (LVP), highly susceptible and highly refractory to *B. malayi* infection, were selected from the original susceptible LVP strain using pairwise mating strategies. For the initial selection, five females and one male were placed together in a 0.4-liter paper carton. The females then were exposed to anesthetized *B. malayi*-infected jirds, *Meriones unguiculatus,* with microfilaremias of 264 or 268 mf/20 μl blood. Engorged females were maintained individually in glass vials (15 ml) covered with marquisette. Strips of filter paper (10 × 50 mm) were placed in each vial and 1 ml of double-distilled H_2O was added. After oviposition, the paper strips with eggs were removed, labeled, dried at room temperature, and stored in separate vials until the female parent's infection status was determined. Twelve days following *B. malayi* exposure, the mosquitoes were dissected to determine if infective third-stage larvae were present. Prevalence and mean intensity of infection then were recorded. Ovipositional strips containing the F_1 progeny of those females that were highly susceptible, i.e., high intensity of infective-stage larvae, or refractory, i.e., no infective-stage larvae, to infection were hatched and reared in separate pans. These F_1 adults were sibling mated (i.e., five sisters mated with one brother) and the protocol was repeated as necessary. After obtaining a prevalence of infection >95% for the susceptible substrain (LVP^{sbm}) and <5% for the refractory substrain (LVP^{rbm}) as compared to 84% susceptible for the original LVP population, colonies of each substrain were established. These selected substrains then were reared as previously described (Christensen and Sutherland 1984; Beerntsen *et al.* 1994).

Midgut isolation. The susceptible and refractory substrains of *A. aegypti* were exposed to *B. malayi* by allowing cartons of mosquitoes to feed on anesthetized jirds with microfilaremias of 136–457 mf/20 μl blood for 10–15 min/carton. Next, mosquitoes were CO_2 anesthetized and engorged mosquitoes selected for midgut isolation. Midguts were removed and placed individually in depression slides

containing *Aedes* saline (Hayes 1953). Any dissection in which erythrocytes were noted outside the midgut was discarded (LaFond *et al.* 1985). Slides containing isolated midguts then were placed in an environmental chamber to maintain a constant temperature ($T = 26.5°C$) for midgut penetration. Two hours following isolation, individual midguts were removed from the depression slide, placed in a drop of 2% formalin on a microscope slide, dissected, and allowed to air dry. Later, mf on these slides were counted at 100× using phase contrast optics. Microfilariae remaining in each depression slide were counted with the aid of a dissecting scope.

Anticoagulation studies. To assess if blood coagulation significantly hindered midgut penetration, both substrains of mosquitoes were exposed to heparinized microfilaremic blood through a membrane feeder (Rutledge *et al.* 1964). Blood from a *B. malayi*-infected jird was obtained via heart puncture, mixed with heparin diluted with Hepes buffer (10 units/1 ml blood), and added to a membrane feeder (274 mf/20 μl blood). Susceptible and refractory mosquitoes were exposed to the feeder for 10–15 min, midguts were removed, and midgut penetration was assessed as described above.

Reciprocal inoculations. After midgut penetration, *B. malayi* mf migrate through the hemocoel and penetrate into the cells of the thoracic musculature, where they molt twice to become infective-stage larvae. Consequently, additional experiments were designed to determine if exposure to the refractory midgut environment compromised the ability of mf to survive and develop into infective larvae within the thoracic musculature. Briefly, cartons of susceptible and refractory mosquitoes were exposed to *B. malayi*-infected jirds (290 or 381 mf/20 μl blood) and midguts were isolated as described earlier. As mf penetrated the susceptible midguts, 10–15 mf were aspirated into a micropipette and intrathoracically inoculated into individual susceptible or refractory females (Lafond *et al.* 1985). This procedure was then repeated, allowing mf to penetrate refractory midguts prior to inoculation into susceptible or refractory females. Ten to 12 days later, the inoculated females were dissected to determine if infective-stage larvae were present. Prevalence and mean intensity were calculated.

Data analysis for mf/midgut parameters. Four separate trials assessing numbers of mf ingested, numbers of mf penetrating the midgut, and percentage of mf penetrating the midgut in the susceptible and refractory mosquitoes were performed. Data were normalized using a square root or arcsine square root transformation and a Student's independent *t* test was performed. Data for the anticoagulation study were treated as described above. Differences were considered significant at $P < 0.05$. Correlation analysis comparing numbers of mf ingested and numbers of mf penetrating the midgut also was performed.

Genetic crosses for assessment of ingestion of mf. We investigated the genetic basis for midgut penetration by mf and for the ability of *A. aegypti* to ingest mf by preparing a F_2 intercross population consisting of 167 individuals from our susceptible and refractory substrains. Five refractory females and one susceptible male were placed together in a 0.4-liter paper carton. Three days later, the females were exposed to an anesthetized, uninfected jird for egg production. Engorged females were maintained individually in glass vials (15 ml) covered with marquisette. Strips of filter paper (10 × 50 mm) were placed in each vial and 1 ml of double-distilled H_2O was added. After oviposition, the paper strips with eggs were removed, labeled, dried at room temperature, and stored in separate vials. Females (parental generation) that had produced eggs then were placed into individual paper cups covered with marquisette, and on the next day exposed to an anesthetized, *B. malayi*-infected jird (182 mf/20 μl blood). Engorged mosquitoes were CO_2 anesthetized and their midguts removed and assessed as described above. After midgut removal, each individual carcass was processed for DNA extraction as previously described (Severson *et al.* 1993). Three individual egg strips from refractory parental females with low permissiveness of midgut penetration (0, 6, or 10 mf penetrated each midgut when 19, 61, and 87 mf, respectively, were ingested) were hatched and reared in separate pans. Two sets of five adult females and one male from the same F_1 egg paper were placed together in a 0.4-liter paper carton and treated as described above to obtain F_2 egg papers. Egg strips containing F_2 progeny were hatched and reared in separate pans. These F_2 progeny were exposed to *B. malayi*-infected jirds (179–372 mf/20 μl blood), their midguts were isolated, and their phenotype was evaluated relative to number of mf ingested, number of mf penetrating the midgut, and number of mf remaining in the midgut. DNA was extracted from the individual F_2 carcasses and processed as previously described (Severson *et al.* 1993).

Linkage analysis and QTL mapping. Multipoint linkage analysis and QTL mapping were conducted using the MAPMAKER/QTL computer package (Lander *et al.* 1987). This package employs the method of interval mapping for QTL (Lander and Botstein 1989). A lod score of 2.0 was used as a threshold for QTL determination. Recombination frequencies were converted into centimorgan map distances using the Kosambi function (Kosambi 1944). QTL position was identified following a previously described format (Severson *et al.* 1994). The general linear models procedure of SAS (SAS Institute, 1990) was used to evaluate genotype by phenotype interactions with QTL markers. The QTL analysis was limited to chromosome 2 only, because no informative RFLP markers were identified for chromosomes 1 and 3. A composite linkage map for chromosome 2 using data from several populations was constructed as previously described (Severson *et al.* 1995).

Results

Substrain selection. The infection prevalences for the selected substrains were >95% for the susceptible and <5% for the refractory as

TABLE I
Microfilariae Ingestion and Midgut Penetration Values for Susceptible (S) and Refractory (R) *Aedes aegypti* Exposed to *Brugia malayi*-Infected Jirds

Mf[a]	Strain (n)	No. ingested ($X \pm$ SD)	P^b	No. penetrated ($X \pm$ SD)	P^b	No. remaining ($X \pm$ SD)	P^b	% penetrated ($X \pm$ SD)	P^c
136	S (22)	50 ± 48	0.447	32 ± 39	0.070	18 ± 21	0.025	53 ± 29	0.011
	R (13)	45 ± 25		16 ± 20		29 ± 16		29 ± 30	
249	S (13)	225 ± 166	0.010	164 ± 128	0.001	61 ± 44	0.086	69 ± 16	0.001
	R (13)	96 ± 68		5 ± 4		91 ± 65		5 ± 4	
307	S (17)	138 ± 91	0.001	99 ± 72	0.001	39 ± 43	0.270	65 ± 29	0.001
	R (17)	55 ± 33		16 ± 22		39 ± 17		23 ± 20	
457	S (19)	286 ± 181	0.071	215 ± 139	0.010	71 ± 63	0.001	72 ± 22	0.001
	R (23)	379 ± 219		123 ± 103		256 ± 172		31 ± 22	

[a] Microfilaremias (mf/20 μl blood) of *B. malayi* jirds.
[b] Student's independent t test on square root transformed data.
[c] Student's independent t test on arcsine square root transformed data.

compared to 84% for the susceptible LVP parental strain. The susceptible substrain resulting from the selection strategy had a higher intensity of infection (15.0 infective-stage larvae (L_3s)/infected mosquito) when exposed to the same concentration of microfilariae as compared to the parental LVP susceptible strain (6.5 L_3s/infected mosquito). The susceptible and refractory substrains are currently in their 26th generation without selection and have infection prevalences of 93.4% (N = 91) and 2.0% (N = 100), respectively, with a mean intensity of 13.4 L_3s/infected mosquito for the susceptible and 1 L_3/infected mosquito for the refractory substrain.

Ingestion of mf and midgut penetration by mf. A substrain related difference in numbers of mf ingested was observed in two of the trials, with the refractory substrain ingesting significantly fewer mf than susceptible mosquitoes (Table I). While a strong correlation between numbers of mf ingested and numbers of mf penetrating the midgut was noted in the susceptible substrain (r^2 = 0.94), a much weaker correlation between these factors was observed in refractory mosquitoes (r^2 = 0.62), suggesting that variables other than number ingested considerably influence midgut penetration in this strain.

Differences in the permissiveness of the midgut for penetration were evident in three of four trials; i.e., significantly fewer mf penetrated refractory as compared to susceptible midguts (Table I). At the lowest microfilarial density (136 mf/20 μl blood), penetration values were not significantly different between the two substrains. However, there were significant differences in the number of mf remaining in the midgut and in the percentage of mf penetrating

TABLE II
Microfilariae Ingestion and Midgut Penetration Values for Susceptible (S) and Refractory (R) *Aedes aegypti* Exposed to Heparinized *Brugia malayi*-Infected Blood

Mf/20 μl[a]	Strain (n)	No. ingested[b] ($X \pm$ SD)	No. penetrated[b] ($X \pm$ SD)	% penetrated[c] ($X \pm$ SD)
274	S (18)	61.6 ± 19.9	40.2 ± 24.7	59.6 ± 25.4
	R (16)	46.7 ± 24.1	19.5 ± 20.1	37.5 ± 31.7

[a] Microfilaremia of blood.
[b] Significantly different with $P < 0.05$ by Student's independent t test on square root transformed data.
[c] Significantly different with $P < 0.05$ by Student's independent t test on arcsine square root transformed data.

TABLE III
Prevalence and Mean Intensity of L_3 *Brugia malayi* in Susceptible (S) and Refractory (R) *Aedes aegypti* Inoculated with Microfilariae That Had Penetrated S or R Midguts

Donor midgut	Recipient mosquito	Prevalence % (No. infected/No. examined)	Mean intensity (No. L_3/Infected mosquito)
S	S	95.7 (22/23)	2.91
R	S	83.3 (20/24)	3.1
R	R	0 (0/25)	—
S	R	12.8 (6/47)	1.83

the midgut, indicating that midgut penetration occurs more readily in susceptible as compared to refractory mosquitoes (Table I). Although number of mf ingested may fluctuate due to environmental factors, the percentage of ingested mf that penetrate the midgut was always significantly greater in susceptible as compared to refractory mosquitoes (Table I).

Anticoagulation studies. As was seen in the previous midgut studies, significantly fewer mf were ingested and significantly fewer penetrated the midgut in refractory as compared to susceptible mosquitoes, suggesting that a lack of anticoagulant in the saliva of refractory mosquitoes was not responsible for differences observed in midgut penetration and ingestion values (Table II).

Reciprocal inoculations. Mf penetrating susceptible midguts developed successfully when inoculated into susceptible mosquitoes, and mf penetrating refractory midguts did not develop when inoculated into refractory mosquitoes (Table III). Both of the other interactions (mf penetrating susceptible midguts and inoculated into refractory mosquitoes and mf penetrating refractory midguts and inoculated into susceptible mosquitoes) suggest that if mf are successful in migrating to the hemocoel, then the suitability of the hemocoelic environment or thoracic musculature determines if development to infective-stage larvae will occur (Table III).

QTL determination. We identified a clear association between QTL genotype and number of mf ingested and number of mf penetrating the midgut (Table IV). However, midgut penetration by mf cannot be independently evaluated for QTL analysis, because the number of mf that can penetrate the midgut is highly correlated with the number of mf ingested with this population. Using RFLP markers, we identified a QTL on chromosome 2 associated with number of mf ingested (Fig. 1). A QTL plot of lod scores for number of mf ingested identified a QTL, designated idb[2,LF181], at the interval

TABLE IV
Association of QTL Genotype with Total Number of Microfilariae Ingested (TOT), Number of Microfilariae Penetrating the Midgut (PEN), and Number of Microfilariae Remaining in the Midgut (MID)

QTL genotype		idb phenotype[a]		
idb [2,LF181][b]	*n*	TOT[c]	PEN[d]	MID[e]
S[f]	32	189.6 ± 145.1	131.8 ± 124.1	57.8 ± 63.0
H	93	148.9 ± 117.1	97.5 ± 90.5	53.5 ± 51.3
R	42	101.7 ± 61.3	40.3 ± 49.6	61.4 ± 54.8

[a] Estimates are given as mean ± SD.
[b] Data are for the LF181 locus.
[c] $P < 0.01$; [d] $P < 0.0001$; [e] not significant. The test statistics represent the results of single factor analysis of variance following the square root transformation of all phenotypic data.
[f] S, RFLP marker homozygous for the susceptible parental genotype; H, marker heterozygous; R, marker homozygous for the refractory parental genotype.

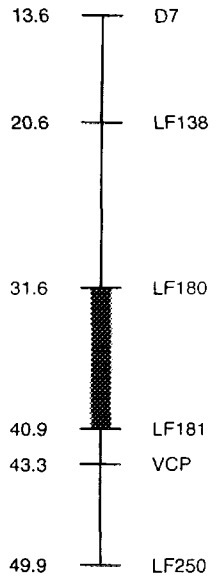

FIG. 1. RFLP genetic linkage map for *Aedes aegypti* chromosome 2 indicating markers used for identification of a QTL associated with idb (intensity determinant for *Brugia malayi*). The interval carrying the putative QTL is identified by a solid bar. Map distances are listed in Kosambi centimorgans.

defined by LF181 and LF180 on chromosome 2. The maximum lod score was 2.3 and the phenotypic variance explained by this QTL was 6.8%.

DISCUSSION

Environmental factors such as pool feeding vs capillary feeding and microfilarial density in the host can significantly influence the numbers of mf ingested; i.e., even when mosquitoes of the same strain feed on the same host at the same time, differences in the number of mf ingested have been observed (Gordon and Lumsden 1939; Obiamiwe 1977). Despite these environmental factors, a substrain-related difference was observed in the number of mf ingested. In four of the six experiments (Tables I, II, and IV), refractory mosquitoes ingested significantly fewer mf than susceptible mosquitoes.

Although differences in the number of mf ingested can influence intensity of infection, midgut suitability impacts both prevalence and mean intensity by affecting the number of mf able to leave the midgut and reach their site of development within the thoracic musculature. A previous study with *Aedes trivittatus* and *Brugia pahangi* has demonstrated that the midgut can function as a barrier to filarial worm infection by denying access to the hemocoelic environment (Sutherland et al. 1986). In that study, only 7.5% of *B. pahangi* mf ingested by *A. trivittatus* penetrated through the midgut within 2 hr postexposure. The influence of the midgut in the present studies also was quite profound in some instances. For example, in 3 cases over 250 mf were ingested by a refractory mosquito, but no mf were able to penetrate through the midgut. Overall, 16.7% (11/66) of the refractory midguts did not permit mf penetration. Therefore, while a mosquito may have a susceptible genotype that is favorable to larval development, it can ultimately be refractory to infection if mf are unable to penetrate through the midgut, indicating that midgut suitability can directly influence susceptibility.

Results from reciprocal inoculations further suggest that the genotype of the hemocoel or thoracic musculature influences susceptibility if mf can penetrate the midgut. Because the selected substrains were not completely susceptible or refractory to filarial worm infection, alleles for susceptibility or refractoriness were present in each strain. Therefore, while most susceptible recipient mosquitoes were infected following inoculation, regardless of whether mf penetrated a susceptible or refractory midgut, a few (5/47) susceptible mosquitoes were not infected, likely due to poor inoculations or the presence of refractory alleles in these particular mosquitoes. The presence of susceptible alleles in a few refractory recipient mosquitoes (6/72) could account for the low prevalence observed in those recipient mosquitoes inoculated with mf that penetrated a susceptible midgut. In 5 of the 6 refractory recipient mosquitoes in which development occurred, only one infective-stage larva was found, but in the sixth mosquito, six larvae were observed.

Analysis using QTL mapping identified a QTL on chromosome 2, idb[2,LF181], that

seems to be primarily an influence on number of mf ingested. Although other QTL influencing mf ingested may exist, our F_2 mapping population lacked the unique marker polymorphisms on chromosomes 1 and 3 that would be required to assess this possibility. That is, while RFLP unique to each substrain were identified for loci on these chromosomes, at least one RFLP fragment was common to both substrains at each locus. Specific allele frequencies observed with both substrains for loci encompassing the entire *A. aegypti* genome are presented elsewhere (Severson and Kassner 1995). Because midgut penetration by mf is not an independent trait (i.e., it is highly correlated with numbers of mf ingested), we were unable to map this trait. However, since our other studies clearly indicate a significant difference in the ability of mf to penetrate the midgut, we believe that at least one additional gene is involved in determining the permissiveness of the midgut for penetration by mf. We are, however, unable to define the presence of, or genomic location for, this locus with our RFLP mapping population.

Of significant interest is that idb[2,LF181] encompasses the same general genome region previously associated with a QTL for susceptibility to *B. malayi*, fsb[2,LF98] (Severson *et al.* 1994). This suggests that those factors influencing the suitability of the midgut for filarial worm penetration and ingestion ability might represent the phenotypic expression of one or more of the genes influencing *B. malayi* susceptibility in *A. aegypti*. Also located in this same region of chromosome 2 is a QTL for susceptibility to the malarial parasite, *Plasmodium gallinaceum*, pgs[2,LF98] (Severson *et al.* 1995), and the PGM isozyme locus that has been correlated with a putative locus influencing yellow fever virus susceptibility (Tabachnick *et al.* 1985). Our present studies provide further support for the suggestion by Severson *et al.* (1995) that this particular genome region contains either a tightly clustered set of independent genes or a single locus influencing susceptibility of mosquitoes to a variety of pathogens that are ingested and then must penetrate the midgut in order to establish a successful infection. It is interesting to note that data involving susceptibility of *A. aegypti* to the filarial worm, *Dirofilaria immitis*, a parasite that develops within the Malpighian tubules and does not penetrate the midgut, provide no evidence of any QTL for susceptibility to *D. immitis* infection in this region of chromosome 2 (Severson, unpublished).

In conclusion, our data show that ingestion of mf influences intensity of infection and that the permissiveness of the midgut for penetration influences not only this parameter but also prevalence of infection. For example, if mf cannot penetrate the midgut, a susceptible genotype in the hemocoel or thoracic environment is irrelevant. Furthermore, genetic mapping of the numbers of parasites ingested identified a QTL on chromosome 2 that is in a region that contains one or more genes that influence the susceptibility of *A. aegypti* for penetration by several mosquito-borne pathogens that need to traverse the midgut in order to establish a successful infection. These findings could have significant value because they delineate potential mechanisms of vector competence that can influence several different mosquito-borne pathogens.

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