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Inbreeding depression affects life-history traits but not infection by *Plasmodium gallinaceum* in the Asian tiger mosquito, *Aedes albopictus*

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ABSTRACT

Emerging and re-emerging vector-borne diseases represent an increasingly significant public health challenge. While geographic variation among populations of vector species for susceptibility to pathogen infection and vector competence has been thoroughly documented, relatively little attention has been devoted to understanding the ultimate evolutionary causes of this intraspecific variation. Local genetic drift is known to influence genetic differentiation among populations for a variety of container-inhabiting mosquito species, including *Aedes albopictus*. Because genetic drift is expected to reduce genetic variation and lead to the accumulation of (partially) recessive deleterious alleles, we hypothesized that reduced genetic variation might affect susceptibility to pathogen infection in a model pathogen-vector system. We therefore created replicate inbred (two generations of full-sib mating, expected $f = 0.375$) and control (expected $f \approx 0.07$) lines of *Ae. albopictus* and measured life-history traits including larval survivorship, adult longevity, and female wing length (body size) as well as susceptibility to infection by a model pathogen, *Plasmodium gallinaceum*. Inbred mosquitoes had significantly reduced larval survivorship and female adult longevity but inbreeding did not affect male adult longevity or female wing length (body size). Furthermore, there was no effect of inbreeding on susceptibility to infection by *P. gallinaceum*. Therefore, while our results did not support the hypothesis that reduced genetic variation influences susceptibility to pathogen infection in this system, we did find evidence for an effect of reduced genetic variation on female adult longevity, an important component of vectorial capacity. We suggest that additional research is needed to elucidate the genetic underpinnings of intraspecific variation in traits related to disease transmission and discuss the implications of our results for the efficacy of creating transgenic strains refractory to disease transmission.

1. Introduction

The re-emergence of mosquito-borne diseases including malaria, yellow fever, and dengue fever represents one of the most significant public health challenges of the 21st century (Breman, 2001; Guzman and Kouri, 2002; WHO, 2002; Barrett and Higgs, 2007). The re-emergence of these diseases is due to complex interactions amongst sociological, ecological and evolutionary factors. For example, invasions by novel vector species may shift the ecological dynamics of a community and alter the prevalence of vector-borne disease (Juliano and Lounibos, 2005). Additionally, the evolution of pesticide resistance in mosquito vectors (Hemingway and Ranson, 2000) and drug resistance in pathogens (Wellems and Plowe, 2001) have clearly led to increased vector abundance and disease prevalence. While a great deal of progress has been made in understanding the physiological and molecular bases of vector-pathogen interactions (Dimopoulos et al., 2001; Lowenberger, 2001; Barillas-Mury et al., 2005; Christensen et al., 2005; Michel and Kafatos, 2005), determining the evolutionary forces that influence the ability of vector species to transmit pathogens has received less attention. This is surprising given the clear role of evolutionary processes in causing current patterns of re-emerging infectious disease noted above.

Vector competence is defined as the ability of a vector to become infected by and subsequently transmit a pathogen (Higgs and Beaty, 2005). It is well established in mosquitoes that vector competence is at least partially genetically determined, and can vary due to differences in a mosquito's susceptibility, infection, dissemination and transmission efficiency (Beerntsen et al., 2000; Higgs and Beaty, 2005). Inherent physiological characteristics, such as the midgut and transmission barriers (i.e., sites that can impede pathogen progress), may reduce a mosquito's susceptibility to viruses, *Plasmodium* and other pathogens (Grimstad and Walker, 1991; Beerntsen et al., 2000; Vinetz et al., 2000; Bennett

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et al., 2002; Black et al., 2002; Higgs and Beaty, 2005). Additionally, mosquito immune responses, including the up-regulation of antimicrobial peptides (Beerntsen et al., 2000) and the melanization response (Paskewitz and Riehle, 1994; Nayar and Knight, 1999; Shiao et al., 2001; Hillyer et al., 2003; Barillas-Mury et al., 2005; Christensen et al., 2005), have been shown to inhibit bacterial, filarial worm and *Plasmodium* proliferation. More recently, it has been demonstrated in *Aedes aegypti* that the RNA interference machinery can inhibit viral replication (Sanchez-Vargas et al., 2009).

A series of elegant studies have demonstrated that ecological factors such as larval competition (Alto et al., 2005, 2008a; Bevins, 2008), pre-existing pathogen infection (Paulson et al., 1992; Comiskey et al., 1999), nutritional stress (Comiskey et al., 1999; Vaidyanathan et al., 2008), and temperature (Westbrook et al., in press) can affect the vector competence of mosquitoes. Nutrient manipulation studies have also found that pathogen susceptibility or transmission may be positively (Lyimo and Koella, 1992; Sumanochitrapon et al., 1998; Okech et al., 2007) or negatively (Grimstad and Haramis, 1984; Grimstad and Walker, 1991; Paulson and Hawley, 1991; Alto et al., 2008b) correlated with adult size, depending on the population and species examined.

However, it is also known that geographically distinct populations of mosquito vectors maintained under standardized (i.e., "common garden") laboratory conditions often exhibit substantial variation in susceptibility to pathogen infection and transmission. For example, populations of *Ae. albopictus* (Gubler and Rosen, 1976; Boromisa et al., 1987) and *Ae. aegypti* (Gubler et al., 1979; Sumanochitrapon et al., 1998; Failloux et al., 1999; Bennett et al., 2002; Black et al., 2002; Failloux et al., 2002) vary in susceptibility and vector competence for dengue viruses. Populations of *Ae. aegypti* have also been shown to vary in vector competence of yellow fever virus (Black et al., 2002), as have populations of *Ae. polynesiensis* for filarial pathogens (Failloux et al., 1995) and *Culex* species for West Nile virus (Vaidyanathan and Scott, 2007; Reisen et al., 2008). These studies thus demonstrate genetically based differences among populations in susceptibility to pathogen infection and/or the ability to transmit pathogens, indicating that in addition to ecological factors discussed above, evolutionary forces must also contribute at least in part to this among-population variation. However, the evolutionary causes of this variation have received very limited theoretical and empirical attention (but see Lambrechts et al., 2009).

Random genetic drift is known to influence genetic differentiation among populations for a variety of container-inhabiting mosquitoes (Black and Tabachnick, 2005). Based on studies of neutral molecular markers, random genetic drift has been implicated as an important factor mediating population differentiation for the container-inhabiting mosquitoes *Culex pipiens* (Chevillon et al., 1995), *Ae. aegypti* (Bosio et al., 2005; Scarpassa et al., 2008), and *Ae. albopictus* (Black et al., 1988a,b; Kambhampati et al., 1990, 1991; O'Donnell and Armbruster, 2009). For example, F_{st} values are as high as 0.12 for *Ae. polynesiensis* (Failloux et al., 1997), 0.25 for *Ae. albopictus* (de Oliveira et al., 2003), and 0.39 for *Ae. aegypti* (Bosio et al., 2005). In *Ae. albopictus*, line-cross experiments have corroborated the results of molecular studies by showing F_1 heterosis for fitness (r') between Florida populations separated by as little as 98 km (O'Donnell and Armbruster, 2009). These results thus imply that even in nearby populations, local random genetic drift and restricted gene flow have led to the accumulation of alternative (partially) deleterious alleles affecting fitness, although overdominance could also contribute to this effect. Black et al. (1988a,b) suggested that for *Ae. albopictus*, high local population structure was likely the consequence of genetic drift during local population establishment of discrete habitat patches by a small number of individuals or local mosquito control efforts that caused a reduction in population size.

In this study, we tested the hypothesis that inbreeding would lead to an increased susceptibility to pathogen infection in the Asian tiger mosquito, *Ae. albopictus*. Our rationale was that consanguineous mating would exaggerate the effects of local drift causing the accumulation of (partially) deleterious recessive alleles (Lynch et al., 1995), and thereby provide a test of the most extreme conditions under which local genetic drift might affect susceptibility to pathogen infection in a natural population. Populations with reduced genetic diversity due to inbreeding or population bottlenecks have reduced fitness (Charlesworth and Charlesworth, 1987; Hedrick and Kalinowski, 2000) and are more susceptible to pathogen infection across many taxa (O'Brien and Evermann, 1988; Stevens et al., 1997; Coltman et al., 1999; Spielman et al., 2004; Pearman and Garner, 2005; Acevedo-Whitehouse et al., 2006; Calleri et al., 2006; Luong et al., 2007; Ilmonen et al., 2008), including a diverse group of insects (Stevens et al., 1997; Spielman et al., 2004; Calleri et al., 2006; Luong et al., 2007; but see Gerloff et al., 2003; Rantala and Roff, 2006). However, no prior studies have explicitly tested how reduced genetic variation influences the infection susceptibility of vectors.

Ae. albopictus is an invasive, container-inhabiting mosquito that was introduced into North America from Japan in 1985 and spread rapidly across the eastern United States from Texas to southern Florida, New Jersey, Ohio, and Illinois (Hawley et al., 1987; Moore, 1999). *Ae. albopictus* is an aggressive biter of humans (Richards et al., 2006) capable of transmitting a wide variety of arthropod-borne diseases including dengue, eastern equine encephalitis (Gratz, 2004), West Nile (Turell et al., 2001a,b; Holick et al., 2002) and chikungunya viruses (Angelini et al., 2007; Pages et al., 2009). In order to test the effect of inbreeding on life-history and infection susceptibility in *Ae. albopictus*, we measured larval survivorship, adult longevity, female wing length (body size) and susceptibly to infection by *Plasmodium gallinaceum* in replicate control (expected $f \approx 0.07$) and inbred (expected $f = 0.375$) lines. We hypothesized that the effects of inbreeding would differ for male and female adult longevity because longevity may be under differential selective pressure in males vs. females. Although *Ae. albopictus* are not commonly infected with *Plasmodium* species in nature (but see Ejiri et al., 2008), infection of aedine mosquitoes by *P. gallinaceum* has been used extensively as a model system to elucidate factors related to the genetic basis of mosquito immunity and vector competence (e.g. Thathy et al., 1994; Hillyer et al., 2003; Morlais et al., 2003; Alavi et al., 2004; Boete et al., 2004). We predicted that inbred lines of *Ae. albopictus* would have reduced larval survivorship, reduced adult longevity, reduced female wing length (body size) and elevated infection levels relative to non-inbred control lines.

2. Materials and methods

Laboratory husbandry. In order to establish a laboratory colony ("New Jersey"), approximately 1000 *Ae. albopictus* larvae were collected from at least 20 discarded tires located at a tire-recapping facility in Salem, New Jersey (39°35′N, 75°29′W).

Ae. albopictus have been established in Salem, NJ since 1995 (CDC, 2005) and this population has a relatively high fitness compared to other North American populations (O'Donnell and Armbruster, 2009). Mosquitoes were reared under standardized laboratory conditions at 21 °C and 80% relative humidity for six generations with at least 50 males and 50 females in each generation as described in Armbruster and Conn (2006).

In order to generate replicate inbred lines with an expected inbreeding coefficient of $f = 0.375$, we performed two generations of full-sib mating as described in Fig. 1. New Jersey F_7 larvae were