Artificial Selection for Different Host Preferences in Culex pipiens pallens (Diptera: Culicidae) Mosquitoes

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ABSTRACT Most mosquito species display host preferences that are a crucial determinant of the transmission rate of vector-borne pathogens. Although a transgenic approach, based on driving genes for zoophilic into vector populations, has been advocated as a malaria control strategy by the World Health Organization since 1982, the genes involved in mosquito host choice remain poorly understood. Culex pipiens pallens Coquillett mosquitoes were artificially selected for two different host preferences in a specially designed experimental enclosure. Of 3,035 mosquitoes obtained from larvae and pupae collected from the wild (the F0 generation), 27% preferentially fed on pigeons and 16% fed on mice. Following artificial selection for these host preferences over successive generations, the percentage of mosquitoes that preferred to feed on pigeons or mice gradually increased, eventually stabilizing at ~55 and 34%, respectively, after the sixth generation. Intergenerational differences in host preferences were significant (P < 0.001). Furthermore, differences in host preferences between mosquitoes selected to prefer pigeons and those selected to prefer mice were both significant and consistent over almost six generations.

KEY WORDS Mosquito, host, selection, Culex pipiens pallens, bird

The females of most mosquito species require vertebrate blood protein for egg production. Many of these species are medically important because they can feed on humans and, therefore, have the potential to transmit pathogens. Generally, most of mosquitoes exhibit a degree of plasticity in their host preference (Lehane 2005). For example, Culex pipiens quinquefasciatus Say feeds mainly on birds and less frequently on humans (Dixit et al. 2001, Samuel et al. 2004, Zinser et al. 2004). Culex tarsalis Coquillett is primarily ornithophagous, but also occasionally feed on mammalian hosts (Reisen and Reeves 1990, Cooperband et al. 2004).

Different host preferences may occur within the same species. For example, although 85% of Anopheles sinensis Wiedemann mosquitoes collected in cow sheds in Fujian Province, China, preferred to feed on cows, 0.7% preferred to feed on humans (Zhen et al. 1996). Similarly, although 20% of An. sinensis mosquitoes sampled from human dwellings in Guizhou Province, China, preferred to feed on humans, 46% preferred cows (Xiong et al. 1997). Diatta et al. (1998) and Duchemin et al. (2001) showed that natural populations of Anopheles gambiae s.s. can show varying degrees of anthropophily, challenging the notion that An. gambiae s.s. should be considered strictly anthropophilic.

It has been argued that such variations are mainly driven by environmental factors (Zwiebel and Taken 2004). However, like any other phenotypic trait, host preferences presumably can evolve (Gillies 1964, Futuyma 1998). Different populations of An. sinensis or An. gambiae in different environmental conditions may experience divergent selective pressures for different host preferences. If so, variation in host preferences within the same species should be able to be intensified by artificial selection over successive generations in the laboratory. Such an experiment would be useful because it could help identify the genes controlling host choice in mosquitoes. Here, we report the results of artificial selection for different host preferences in Culex pipiens pallens Coquillett.

Materials and Methods

Mosquitoes. Larvae and pupae of Cx. pipiens pallens were collected from a village in southern Beijing in July 2006. Larvae were fed a mixture of yeast powder, wheat and bean flour, and pork liver powder. Pupae were collected daily and kept in mesh-covered cages containing 8% sucrose solution applied to a sponge. Emergent mosquitoes were kept at 70–80% relative humidity (RH), 25–28°C, and a photoperiod of 14:10 (L:D) h. Four- to five-day-old adult female mosquitoes that had not yet had access to a blood meal were randomly collected 6–8h before the start of experiments. In total, 3,035 mosquitoes from this F0 generation were subject to artificial selection for
different host preferences. In addition, the behavioral responses of 7- to 14-d-old, nonblood-fed, mated female mosquitoes to five chemical components isolated from pigeon and mouse odors were tested.

**Host Animals.** Pigeons (white meat pigeon) were purchased from a bird market and fed on a mixture of maize, peas, foxtail millet, and bird grit. Mice (Kuming mice) were provided by the Beijing Institute of Microbiology and Epidemiology’s animal laboratory and fed on a special food provided by the animal laboratory. Food and water were available to both species ad libitum.

**Experimental Enclosure.** We designed an experimental enclosure to distinguish mosquitoes with different host preferences. The enclosure was made of organic glass and consisted of a hexahedral flight chamber (50 cm in height, 30 cm in length) connected to three selection chambers (30 by 30 by 30 cm), with a holding chamber (30 by 30 by 30 cm) at the bottom of the flight chamber (Fig. 1). A funnel made of gauze was placed at the junction of the flight chamber and selection chambers to prevent mosquitoes returning from the selection chambers to the flight chamber. A bezel placed at the connection of the flight chamber and the holding chamber was used to regulate the movement of mosquitoes between these two sections of the apparatus.

**Olfactometer.** A glass, dual-choice Y-tube olfactometer, based on the design and description of Geier et al. (1999), was used in behavioral bioassays. (Fig. 2) The arms of the olfactometer were 50 cm in length and 10 cm in diameter. The angle between the two arms was 75°. Mosquitoes were released into olfactometer’s main tube and chemicals or samples were placed in 7-cm-diameter glass ports attached to the ends of each branch tube.

**Chemicals Isolated From Pigeon and Mouse Odors.** Five attractive chemicals, including octanoic acid (pigeon), nonanal (pigeon and mice), ammonia (mice), β-myrcene (pigeon), and 4-methylphenol (pigeon), isolated from pigeon and mouse odors to mosquitoes selected for their preference for either pigeons or mice was tested. All chemicals were of >98% purity and were obtained from commercial sources (Alfa Aesar, Ward Hill, MA; or Sigma-Aldrich, St. Louis, MO). With the exception of ammonia, which was dissolved in distilled water, all other chemicals were dissolved in methylene chloride (high-performance liquid chromatography grade).
Experimental Procedures. Selection of Mosquitoes for Different Host Preferences. One pigeon and 10 mice, which were immobile, were randomly placed in two of the three selection chambers of the experimental enclosure with the third chamber left empty to serve as a control. The F0 mosquitoes were transferred to the holding chamber and allowed to acclimate for 15 min before being released into the flight chamber. At 1800 hours local time, the bezel at the junction between the flight and release chambers was opened, allowing mosquitoes to enter the flight and selection chambers. Three h after being released, the mosquitoes in each selection chamber were counted. Mosquitoes that had entered selection chambers containing either a pigeon or mice were allowed to feed and lay eggs. The resultant F1 generation was separated into a "pigeon" (P) and "mouse" (M) strain on the basis of these initial parental feeding preferences. The same selection process was applied to 12 successive generations of each subpopulation. Any P-strain mosquitoes that entered a selection chamber containing mice were not allowed to feed or lay eggs and vice versa.

Behavioral Bioassay. A Y-tube olfactometer was used to investigate the attractiveness of chemicals isolated from host odors to each subpopulation. The air in the olfactometer was conditioned to 70–80% RH and 26–28°C, with an air speed of 0.5–1.0 m/s. Air was humidified and filtered through a series of activated charcoal filters before entering the olfactometer. Light was provided by a 15 W tungsten light bulb suspended above the olfactometer. Light was filtered by a piece of yellow cotton cloth between the bulb and the olfactometer; the light intensity inside the olfactometer was 100–150 lux. All assays were conducted between 1700 and 2100 hours local time.

F12 generation of P- and M-strain mosquitoes were assayed for responses to five chemicals isolated from pigeon and mouse odors. Filter paper strips (50 by 50 mm) were impregnated with 500 l of a test solution containing three different concentrations of each chemical (0.1, 1, or 10 l). After the solvent had evaporated (∼3 min), the treated filter paper strips were randomly placed in either the left or right port of the olfactometer. A filter paper strip treated with 500 l of methylene chloride was placed in the opposite port as a control. Thirty 7–14-d-old female mosquitoes that had not had access to a bloodmeal were used per test. Six replicates of each test were conducted so that the preferences of a total of 180 mosquitoes were assayed for each dosage of each chemical. Treatment and control ports were alternated between replicates of each test. Mosquitoes were allowed to acclimate in the olfactometer for 15 min before being released into the main tube. The number of mosquitoes in each branch of the olfactometer was counted 20 min after their release into the apparatus.

Analysis of the Host Specificity of Each Putative Strain. The percentage of mosquitoes in selection chambers containing either a pigeon or mice was used to evaluate the host specificity of each putative strain. Host specificity was calculated as $T \times 100\% / W$, where $T$ is the total number of mosquitoes trapped in a selection chamber containing a pigeon or mice, and $W$ is the total number of mosquitoes released. Differences in host specificity from the F0 to the F12 generation were analyzed with a chi-square test.

Analysis of Odor Preferences. The percentages of mosquitoes entering the branch of the olfactometer containing a test chemical was used to measure the relative "attractiveness" of the various chemical stimuli presented. Relative attractiveness was calculated as $T \times 100\% / (T + C)$, where $T$ is the total number of mosquitoes entering the branch of the olfactometer containing the test chemical (treatment branch), and $C$ is the total number of mosquitoes entering the control branch. Differences in the percentage of mosquitoes entering the control and treatment branches for different doses of the same chemical were analyzed using a one-way analysis of variance, and least significant difference tests were used to identify the mean percentage of mosquitoes that exhibited significant ($P < 0.05$) differences in attraction to the various test compounds (Rosner 1990).

A paired sample t-test was used to assess the significance of differences in attractiveness of the five chemicals to mosquitoes of the P- or M-strains. Two-sided t-probabilities were calculated to test pair-wise differences between means. Effects were considered to be significant at $P < 0.05$ (Oude Voshaar 1994, Sokal and Rohlf 1998).

Results
Selection of Mosquitoes for Different Host Preferences. The results of 12 generations of artificial selection for different host preferences on the founder population of wild-caught Cx. pipiens pallens are shown in Tables 1 and 2. For clarity, difference analysis of data have been only presented on the F0, F4, F8, and F12 generation (Figs. 3 and 4). Of the 3,035 F0 mosquitoes, 27% preferred pigeons to mice and 16% preferred mice to pigeons. After 12 generations of selection for these initial host preferences, the proportions of the P- and M-strains that preferred to feed on either pigeons or mice had increased to 56 and 34%.

Table 1. Selection and purification of pigeon preference population of Cx. p. pallens

<table>
<thead>
<tr>
<th>Generation of pigeon blood-fed mosquitoes</th>
<th>No. of mosquitoes selected (%)</th>
<th>Rate of pigeon selected (%)</th>
<th>Rate of mouse selected (%)</th>
<th>Rate of no response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>3,035</td>
<td>27</td>
<td>16</td>
<td>57</td>
</tr>
<tr>
<td>F1</td>
<td>2,054</td>
<td>43</td>
<td>23</td>
<td>34</td>
</tr>
<tr>
<td>F2</td>
<td>1,312</td>
<td>27</td>
<td>22</td>
<td>51</td>
</tr>
<tr>
<td>F3</td>
<td>1,781</td>
<td>40</td>
<td>23</td>
<td>37</td>
</tr>
<tr>
<td>F4</td>
<td>592</td>
<td>44</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>F5</td>
<td>858</td>
<td>50</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>F6</td>
<td>1,378</td>
<td>58</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>F7</td>
<td>2,133</td>
<td>56</td>
<td>7</td>
<td>37</td>
</tr>
<tr>
<td>F11</td>
<td>1,898</td>
<td>54</td>
<td>7</td>
<td>39</td>
</tr>
<tr>
<td>F12</td>
<td>2,346</td>
<td>56</td>
<td>8</td>
<td>36</td>
</tr>
</tbody>
</table>

There is no accurate data in F7, F9, and F10 generation of Cx. p. pallens.
respectively. Ten percent of the P-strain continued to prefer to feed on mice and 31% of the M-strain continued to prefer pigeons. Intergenerational differences in host specificity were significant ($P < 0.001$).

Furthermore, differences in host specificity between the two strains were both significant and consistent over almost six generations ($F < 0.001$; Figs 5 and 6). There was a 29% increase in the proportion of the P-strain that preferred pigeons between the F0 and F12 generations, and an 18% increase in the proportion of M-strain that preferred mice over the same number of generations. These results suggest that different host preferences in *Cx. p. pallens* have a genetic basis and can be intensified by selection.

It was worth noting that the percentage of nonresponders gradually decreased from the first trail to the last selection. Although the percentage of nonresponders in the mouse blood-selected strain was higher than that of pigeon blood-selected strain in every generation, the percentage of nonresponders for both strains stabilized at around 35% (Tables 1 and 2).

Relative Attractiveness to Mosquitoes of Five Chemical Components of Pigeon and Mouse Odors. The relative attractiveness of five chemicals isolated from pigeon and mouse odors to mosquitoes from the P- and M-strains is shown in Fig. 7. Of the chemicals and dosages assayed, a significant difference

![Fig. 3. Comparison of proportion of mosquitoes that preferred to feed on pigeons in different generations of *Cx. p. pallens* artificially selected to preferentially feed on pigeons. F0, F4, F8, and F12 are different generations of mosquitoes; the F0 generation were adults raised from larvae and pupae collected in the wild. Values are means ± SE. Significance levels: ***$P < 0.001$; **$P < 0.01$; ns, not significant.](https://academic.oup.com/jme/article-abstract/52/5/1067/828316)

![Fig. 4. Comparison of proportion of mosquitoes that preferred to feed on mice in different generations of *Cx. p. pallens* artificially selected to preferentially feed on mice. F0, F4, F8, and F12 are different generations of mosquitoes; the F0 generation were adults raised from larvae and pupae collected in the wild. Values are means ± SE. Significance levels: ***$P < 0.001$; **$P < 0.01$; ns, not significant.](https://academic.oup.com/jme/article-abstract/52/5/1067/828316)
in attractiveness was apparent for only 10 µl/ml of octanoic acid \((P = 0.007)\) and 0.1 µl/ml of 4-methylphenol \((P = 0.024)\), which were both significantly more attractive to mosquitoes of the P-strain than those of the M-strain.

The behavioral responses of both strains to other dosages of octanoic acid and 4-methylphenol, and to all dosages of ammonia, were quite similar. Chemicals that were attractive to the P-strain were also attractive to the M-strain but to a different degree. However, these differences were not significant \((P > 0.05)\). Nonanal and \(\beta\)-myrcene were either only marginally, or not attractive, to both strains. The repellent or marginal response should be the result of using high dose of chemical.

**Discussion**

Mosquitoes frequently display variation in host preferences between species but also within the same species. Although little is known about the determinants of these host preferences, there is presumably some genetic variation in host choice (Lyimo and Ferguson 2009). The domestic mosquito *Cx. pipiens pallens*, the most widely distributed mosquito in China, is the primary vector of wuchereriasis and epidemic cephalitis (Ye 1995, Rowland et al. 1999). More than 50 yr ago, the host preferences of this species in China were quantified by recording the relative frequency of blood from different hosts species in samples of engorged mosquitoes.
mosquitoes collected in Beijing. It was found to be a generalist feeder, feeding mainly on humans, domestic animals, and poultry (Liu et al. 1959). We found that mosquitoes of this species reared from larvae or pupae obtained from the wild generally preferred to feed on pigeons rather than mice. Field experiments indicate that Cx. pipiens pallens, which generally lives in human dwellings, prefers chicks to mice (Zhang et al. 2007). Our results are in agreement with the findings of Zhang et al. in indicating that natural populations of Cx. pipiens pallens in the Beijing area generally prefer to feed on avian hosts.

However, after 12 generations of artificial selection the proportion of the population that preferred pigeons to mice gradually increased from ~27% to ~56%. Furthermore, a higher proportion of the resultant P-strain preferred feeding on pigeons than did the M-strain and vice versa. Although the proportion of either strain that preferred feeding on pigeons or mice did not reach 100%, host preferences tended to be stable after 12 generations.

Meanwhile, the results showed the percentage of nonresponders decreased from 57% of the first trial to ~35% after 12 generations, indicating that the experiment put the wild generation through a selective bottleneck. Subsequently, the percentage of nonresponders in the M-strain was higher than that of P-strain, indicating that they went through a stronger selective bottleneck than the pigeon blood-selected one.

These results indicate that host preferences in Cx. pipiens pallens have a genetic basis. Within very few generations, we were able to produce strains that differed significantly in their preference for either pigeons or mice. Moreover, only after 12 generations of directional selection, there was a more significant response to the chemicals (octanoic acid and 4-methylphenol) isolated from pigeon odor in Cx. pipiens pallens strain selected by pigeon blood than in strain selected by mice. Our results provide strong evidence that there is genetic variation in host species preferences within generalist mosquito populations. More than 40 yr ago, Gillies (1964) showed that several generations of laboratory selection could change the feeding preferences of a wild anthropophilic population of An. gambiae from humans to calves. Lefèvre et al. (2009) suggested that simply rearing these mosquitoes in the laboratory, where they are generally fed on nonhuman animals, may have caused individual mosquitoes to modify their innate host preferences. However, we think it more likely that a minority of the wild population preferred calves to humans and that this proportion was increased by several generations of laboratory selection for individuals that would readily feed on calves.

In our experiments, the proportion of the P- or M-strains that preferred pigeons or mice stabilized at 55 and 34%, respectively, from the 6th generation to the 12th generation. These results indicate that mosquito host choice, like other phenotypic characteristics, is stable and can be transmitted to future generations. This conclusion is supported by work by Zhang et al. (2007), who consistently fed a laboratory strain of Cx. pipiens pallens on mouse blood. However, when given...
a choice, this strain preferred chick and pigeon blood to mouse blood (Zhang et al. 2007). In other words, without active selection, the innate host preference of the natural population remained unchanged. These findings indicate that mosquito host preferences are regulated by genes but further research is required to identify the specific genes responsible.

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