ENHANCED DEVELOPMENT IN NATURE OF LARVAL ANOPHELES ARABIENSIS MOSQUITOES FEEDING ON MAIZE POLLEN

YEMANE YE-EBIYO, RICHARD J. POLLACK, AND ANDREW SPIELMAN

Abstract. To determine whether pollen produced by maize (Zea m. mays) may contribute to the development of larval Anopheles gambiae complex mosquitoes, the main African vectors of malaria, we correlated duration of larval development, pupation success, and size of the resulting adults with degree of access to this potential nutrient. Maize pollen is abundant during the wet season on the surface of water near maize plantings in a malaria-endemic region of Ethiopia, and larval Anopheles arabiensis readily ingest these particles in nature. Larvae develop to the pupal stage more rapidly, more frequently, and produce larger adults where maize pollen is abundant than do those that have little access to this food. The force of transmission of malaria in sub-Saharan Africa might be reduced if maize plantings were excluded from the immediate vicinity of homes or, perhaps, if pollen of such maize were to express entomotoxins.

INTRODUCTION

Those members of the Anopheles gambiae complex of African mosquitoes that serve most efficiently as vectors of malaria develop in transient bodies of turbid water that generally are vegetation-free. Other anophelines, such as Anopheles quadrimaculatus, generally develop in relatively clear water by exploiting the bacteria-rich surface environment of permanent marshes through their interfacial feeding strategy. Suspended microorganisms and detritus comprise the main food for various larval Culex mosquitoes, and many Aedes mosquitoes browse on adherent or sedimented organic materials. The feeding strategies used by mosquitoes that breed in turbid transient water, however, remain unexplored. The surfeit of inert particles in the larval habitats of Anopheles arabiensis suggests that their feeding strategy may be adapted peculiarly to this disturbed environment.

Although pollen might provide a crucial nutritional resource for surface-feeding anophelines, such food would be scarce in sub-Saharan Africa because tropical plants generally are vegetation-free. However, maize (Zea m. mays), however, has become ubiquitous during the rainy season in much of tropical Africa, and this crop appears to provide a prolific source of wind-borne pollen. Maize pollen grains are relatively large (50–90 μ) and thin-walled. Although a few such grains may be carried over great distances by strong gusts of wind, they generally settle to the ground within about 60 meters, and these grains tend to be trapped in the surface film of any body of water on which they settle. Potential anopheline breeding sites that are situated in the immediate vicinity of a maize planting, therefore, would be enriched by this potentially important food source. Maize is perhaps the most common crop grown in the immediate vicinity of homes and is planted throughout a wide range of climates and altitudes in Ethiopia and in many other parts of Africa. Anopheles arabiensis breeds in such peridomestic situations where drainage is disturbed. The borrow-pits used to extract mud for house construction, and the ruts formed by the wheels of motor vehicles around these houses, for example, frequently are exploited by these mosquitoes. In this manner, maize constitutes an integral part of the landscape that includes the human hosts and breeding grounds of these dangerous vectors of malaria.

This consideration stimulated us to ask whether larval mosquitoes of the An. gambiae complex may develop more readily in immediate proximity to flowering maize than in the absence of this prolific source of pollen. In particular, we determined whether larval An. arabiensis might develop to the pupal stage in an Ethiopian village more rapidly, more frequently, and produce larger adults where maize pollen is abundant than where these particles are absent.

MATERIALS AND METHODS

This study was conducted near Zwai, a small town located in the Rift Valley of Ethiopia. Anopheles arabiensis serves as the major vector of malaria there. Other anophelines, including another member of the An. gambiae complex, An. quadrimaculatus, also are present in Ethiopia. This taxon has recently been designated as An. quadriannulatus species B. Of a sample of 20 larvae in the study site examined microscopically and by the polymerase chain reaction, all proved to be An. arabiensis. Cytogenetic studies confirmed that this species is the sole member of the complex found near Zwai and that it breeds mainly in the small collections of turbid water that were the subject of this study. To record the distribution of pollen in the vicinity of maize fields, charged microscope slides (Superfrost/Plus; Fisher Scientific, Pittsburgh, PA) were dipped vertically through the surface of water in natural larval breeding habitats, removed, and dried. Slides were cover-slipped and examined microscopically at 100× to identify adherent particles. To sample air-borne pollen that might settle on bodies of water in the study area, microscope slides covered with a thin coat of silicone vacuum grease were placed horizontally on the ground near several natural breeding sites and experimental pits.

A series of artificial breeding sites were dug and maintained during July and August of 1998 and 1999 in the vicinity of Zwai, located about 100 meters from a field of maize then undergoing anthesis. These circular non-shaded “pits” were each 30 cm deep and 50 cm wide and lined with a plastic sheet covered by a thin layer of soil. Water was taken from the nearby lake and 10 liters decanted into each pit. Larval An. arabiensis, collected from nearby natural breeding sites, served to stock each pit. Of these arti-
ficial breeding sites, some remained open; others were fully enclosed by a tent-like plastic sheet; others were enclosed partially in this manner; and yet others, that remained open, were wiped daily with tissue paper to remove particles entrapped in the surface film. About 0.2 g of maize pollen was sprinkled daily onto the surface of other pits. Others were placed 25 meters from maize plantings and left open. The ratio of pupae to larvae in each pit was determined in five 200-ml samples (taken by means of a standard 300-ml dipper). The water and all larvae were replaced after the number of larvae and pupae in each dip was recorded. A pair of additional circular pits was placed near (10 meters) and distant (50 meters) from a maize field that was then in flower and each was stocked with 40 second-instar larvae harvested from natural breeding sites. Development of larvae to the pupal stage was followed for six consecutive days. The temperature of the water in each pit was measured each day at noon. Supplemental water was added to maintain a constant water level. Other natural breeding sites near (< 50 meters) and far (> 100 meters) from flowering maize plantings were also searched for the presence of larval or pupal An. arabiensis.

The relative pupal productivity of diverse regimens of pollen deletion and substitution was calculated using odds ratios (ORs). Thus, we compared the proportion of larvae pupating under one such regimen to the proportion pupating under the natural ambient regimen. A 95% confidence interval (CI) was calculated.

**RESULTS**

**Preliminary observations.** In a preliminary series of observations, we described the food ingested by larval An. arabiensis, the distribution of maize pollen in their vicinity and the physical appearance of the water in which they breed. Characteristic pollen grains of maize were observed microscopically in the gut contents of larvae sampled from all three natural breeding sites located near maize plantings, and such material formed the vast bulk of ingested material. Maize pollen adhered to charged slides that were dipped through the surface of the water that accumulated near human residences. Such pollen also adhered to silicone-coated slides that were placed on the ground for one day 60 meters distant from maize plants then undergoing desiccation. Although at least 11 other kinds of plants shedding pollen grew nearby most of them were insect pollinated. Only maize pollen was observed in the guts of these mosquitoes, on the surface of the breeding sites, or on the surface of the ground near these sites. Larval An. arabiensis generally were present in water that was rendered opaque by suspended mud and in slightly turbid water, but rarely in clear water. These qualitative observations indicate that maize pollen provides an abundant source of food for larval An. arabiensis developing during the rainy season near maize plantings in this malaria-endemic site.

In another exploratory series of observations, the development of larval An. arabiensis in six natural bodies of water located more than 100 meters from any maize planting was compared to that of larvae found in six other sites located near flowering maize. Although larvae were detected in each of these sites, only one of the six remote sites contained pupae; a total of 18 pupae were found in 100 dipper samples taken from this site. Pupae were present, however, in all six sites located near maize plantings. Larval An. arabiensis seem to accumulate without developing to the pupal stage where no maize plantings exist.

**Pollen access and larval development.** We then determined whether degree of access to pollen might influence larval development to the pupal stage during the six days following introduction of larvae into a series of artificial pits. Almost as many pupae as larvae were discovered in experimental pits located adjacent to fields containing flowering maize (Table 1). About one-tenth as many pupae were seen in similar pits that were deprived of pollen, either by wiping daily with absorbent paper towels or by partial or full enclosure under plastic tents (OR = 23.2, 95% CI = 10.8–49.8). In such fully enclosed pits, far more pupae than larvae developed when fresh pollen had been sprinkled daily on the surface of the water (OR = 4.4, 95% CI = 2.4–7.9). We concluded that access to pollen promotes development of larval An. arabiensis.

The effect of duration of access to supplemental pollen on larval development was explored by beginning similar “ablation-rescue” experiments during the first larval instar and others during the second instar. The experimental pits were placed 100 meters from a maize field. Under ambient conditions, about one-tenth as many pupae were seen as larvae, regardless of the duration of the experiment (Table 2), or when pollen was excluded by means of an enclosure. Pollen supplementation, however, resulted in a marked preponderance of pupae. When supplementation began during the first instar, pupae were as numerous as larvae after three days, which is also 8–10 times higher than the excluded and ambient situations (OR = 10.0, 95% CI = 4.4–23.0 and OR = 8.6, 95% CI = 3.8–19.5). When supplementation began during the second instar, more than twice as many pupae as larvae were found (OR = 2.6, 95% CI = 1.3–4.9 and OR = 2.1, 95% CI = 1.1–3.9). The ratio of pupae to larvae was greater on the third day than on the sixth day of the experiment (0.42 versus 0.08 pupae per larva). Many more larval An. arabiensis develop to the pupal stage when newly hatched larvae have access to maize pollen than when pollen supplementation is delayed.

### Table 1

Effect of excluding and of supplementing pollen on the proportion of larval Anopheles arabiensis maturing to the pupal stage and the size of the resulting adults. Developmental stages were sampled and recorded throughout the following six-day period

<table>
<thead>
<tr>
<th>Access to pollen</th>
<th>No. Larvae</th>
<th>Pupae per larva</th>
<th>Wing length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No supplement</td>
<td>41</td>
<td>9.9</td>
<td>3.1</td>
</tr>
<tr>
<td>Ambient site #1</td>
<td>73</td>
<td>0.7</td>
<td>3.1</td>
</tr>
<tr>
<td>Ambient site #2</td>
<td>89</td>
<td>0.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Removed</td>
<td>83</td>
<td>0.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Excluded partially</td>
<td>95</td>
<td>0.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Excluded fully</td>
<td>23</td>
<td>3.1</td>
<td>3.5</td>
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**Supplemented**

Effect of excluding and of supplementing pollen on the proportion of larval Anopheles arabiensis maturing to the pupal stage and the size of the resulting adults. Developmental stages were sampled and recorded throughout the following six-day period.
Effect of duration of pollen availability on the proportion of larval Anopheles arabiensis maturing to the pupal stage and the size of the resulting adults. Developmental stages were sampled and recorded on the third and the sixth day after pupae first were seen.

Table 2

<table>
<thead>
<tr>
<th>Access to pollen</th>
<th>No. larvae</th>
<th>Pupae per larva</th>
<th>Mean ± SD</th>
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<tr>
<td>Ambient sites</td>
<td>98</td>
<td>0.11</td>
<td>3.1 ± 0.30</td>
<td>116</td>
<td>0.08</td>
<td>1.6 ± 0.16</td>
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<tr>
<td>Excluded</td>
<td>92</td>
<td>0.13</td>
<td>3.2 ± 0.28</td>
<td>110</td>
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<td>1.3 ± 0.20</td>
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<td>Supplemented</td>
<td>24</td>
<td>1.13</td>
<td>3.5 ± 0.22</td>
<td>62</td>
<td>0.42</td>
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</tbody>
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Table 3

Effect of distance from flowering maize plants on the development of Anopheles arabiensis maturing to the pupal stage and the size of resulting adults. First-instar larvae were placed in pits near (10 meters) and distant (50 meters) from any such plants.

<table>
<thead>
<tr>
<th>Proximity to maize</th>
<th>No. pupae after day</th>
<th>Wing length (mm)</th>
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<tr>
<td>Near</td>
<td>80</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Distant</td>
<td>80</td>
<td>23</td>
<td>29</td>
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DISCUSSION

Malaria transmission in Ethiopia is narrowly seasonal. The abundance of adult An. arabiensis begins to increase in July with the onset of the rainy season, reaching maximal levels in August. Malaria transmission commences in September and continues through December. This seasonal burgeoning of vector mosquitoes closely coincides with the period of anthesis in maize. The geographic distribution of malaria in Ethiopia also coincides closely with the distribution of maize culture, a crop that was probably introduced into Africa by the Portuguese during the 16th century. It is also interesting to note that many rural Ethiopians believe that malaria is caused by consuming the stalks of young maize plants. More than one-fifth of the residents of our study site expressed similar beliefs. These observations point towards a potentially causal relationship between the intensity of malaria transmission and maize culture in Ethiopia.

The peridomestic collections of water that are exploited by An. arabiensis tend to be transient because they are small, sun-lit, and vegetation-free, and this imposes an important limitation on the life cycle of these mosquitoes. Indeed, even in relatively stable larval habitats in market gardens of Senegal, only about 20% of anopheline larvae develop to the adult stage. Because such larvae must develop so rapidly, a rich source of nutrient is required, and they must be able to select this food from the nearly opaque suspension of crushed soil that almost invariably characterizes these breeding sites.

Our experiments indicate that maize pollen serves as an important source of larval nutrition for mosquitoes that are adapted to thrive in the transient and turbid rainwater collections usually found close to villages and maize fields of Tropical Africa. Anopheles arabiensis, reared in turbid water in the lab on a diet consisting only of maize pollen, produced adults comparable in size to those fed on lab mosquito chow (4 parts ground rabbit lab chow: 2 parts liver powder: 1 part yeast, as adapted in this laboratory).

A medium-sized maize plant may produce as many as 50 million soft-walled pollen grains, and the density of such pollen in a cornfield may exceed 42 thousand grains/in. Although maize pollen is dispersed by air currents, pollen grains almost invariably sediment within 60 meters of the source. Accordingly, we selected our natural and artificial breeding sites at graded intervals (10, 50, and 100 meters) from maize plots.

Mosquitoes of the same species vary in size, with larger adults tending to survive longest. Access to pollen profoundly influences the size of adult A. arabiensis mosquitoes. Because longevity contributes powerfully to the capacity of an insect to transmit infection, vectorial capacity may relate closely to the dissemination of this crop in Africa. Our study indicates that larvae that had fed on maize pollen produce particularly large adults, and this may carry important implications for the intensity of malaria transmission.

Our finding that maize pollen constitutes an important

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source of larval nutriment for the main member of the An. gambiae complex in Ethiopia, suggests a possible strategy for intervening against the transmission of malaria there, in other parts of sub-Saharan Africa and, perhaps, elsewhere. To the extent that maize pollen is ingested by larvae of the An. gambiae complex of mosquitoes, introduced entomotoxic moieties carried by these particles would usefully reduce the force of transmission of malaria. The pollen of certain varieties of this crop has recently proved to be toxic to lepidopterans, and appropriately modified maize may serve similarly in the case of anopheine mosquitoes. Development of a few larval An. arabiensis into pupae in natural breeding sites remote from maize plantings and in the experimental pits from which pollen was excluded, indicates that pollen is not the sole nutrient available to these insects. The pollen of other plants and certain microorganisms may also provide a source of larval nutriment. The larvae of diverse anophe-line mosquitoes appear to employ different feeding strategies. Even if other plant pollens or microorganisms were ingested by these insects, the predominance of maize in many malaria-endemic sites in Africa and its unique proximity to the breeding sites of the An. gambiae complex, suggests that genetically modified maize plants may provide a locally-applied and potentially potent antimalarial vehicle. Taken together, these observations indicate that malaria transmission in Africa may currently be promoted by peri-domestic maize culture because 1) the pollen of these plants is abundant where An. arabiensis breed; 2) their larvae readily ingest this material; 3) their growth is inhibited when they are denied access to such pollen and 4) promoted when access is enhanced; and 5) their larvae grow most rapidly when maize is flowering. The feeding strategy that permits these vectors of malaria to feed selectively in the turbid water in which they breed remains to be explored.

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REFERENCES