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Biological cost of tolerance to heavy metals in the mosquito *Anopheles gambiae*

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Abstract. The global rate of heavy metal pollution is rapidly increasing in various habitats. *Anopheles* malaria vector species (Diptera: Culicidae) appear to tolerate many aquatic habitats with metal pollutants, despite their normal proclivity for 'clean' water (i.e. low levels of organic matter). Investigations were conducted to establish whether there are biological costs for tolerance to heavy metals in *Anopheles gambiae* Giles *sensu stricto* and to assess the potential impact of heavy metal pollution on mosquito ecology. *Anopheles gambiae s.s.* were selected for cadmium, copper or lead tolerance through chronic exposure of immature stages to solutions of the metals for three successive generations. Biological costs were assessed in the fourth generation by horizontal life table analysis. Tolerance in larvae to cadmium (as cadmium chloride, $CdCl_2$), copper [as copper II nitrate hydrate, $Cu(NO_3)_2$ 2.5 H_2O] and lead [as lead II nitrate, $Pb(NO_3)_2$], monitored by changes in LC_{50} concentrations of the metals, changed from 6.07 µg/L, 12.42 µg/L and 493.32 µg/L to 4.45 µg/L, 25.02 µg/L and 516.69 µg/L, respectively, after three generations of exposure. The metal-selected strains had a significantly lower magnitude of egg viability, larval and pupal survivorship, adult emergence, fecundity and net reproductive rate than the control strain. The population doubling times were significantly longer and the instantaneous birth rates lower in most metal-selected strains relative to the control strain. Our results suggest that although *An. gambiae s.s.* displays the potential to develop tolerance to heavy metals, particularly copper, this may occur at a significant biological cost, which can adversely affect its ecological fitness.

Key words. *Anopheles gambiae*, biological cost, cadmium, copper, heavy metals, lead, tolerance.

Introduction

Urban settings represent potentially permanent hot spots for malaria vector production and malaria transmission in Africa (Trape & Zoulani, 1987; Robert *et al*., 1998; Afrane *et al*., 2004; Matthys *et al*., 2006). Growing evidence suggests that *Anopheles gambiae* Giles *s.s*, the most prolific African malaria vector, is expanding its ecological niche into polluted habitats. Recent studies found *An. gambiae* larvae thriving in a variety of anthropogenic urban water bodies, which

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contained pollution from domestic and/or industrial sewage (Awolola *et al*., 2007; Djouaka *et al*., 2007), including heavy metals in excess of natural loads (Mireji *et al*., 2008). These mosquito larvae appear to have increased their tolerance and possibly developed resistance to the pollutants in their natural habitats.

The adaptation of this mosquito to the urban environment is a real threat that can seriously impact the health of the population. However, environmental changes and subsequent adaptation can have consequences on the biological fitness of the mosquito (Reed *et al*., 2003), especially if inherited resistance to selecting agents such as heavy metals develops (Orr, 1998). In the absence of compensatory secondary mutations (Levin *et al*., 2000), the cost would be reflected in a decline of tolerant individuals in environments devoid of heavy metals; these individuals could be displaced by naïve populations with greater reproductive and growth rates (Agnew *et al*., 2004).

The purpose of this study was to determine if *An. gambiae s.s.* populations exhibit a natural range of tolerances to the toxic effects of heavy metals, or if the degree of tolerance can be increased under selection pressure. We hypothesized that the tolerance to heavy metals we observed in wild *An. gambiae s.s.* populations (Mireji *et al*., 2008) has an adverse effect on the biological fitness of the affected mosquito populations. Results could demonstrate the existence of a genetically controlled mechanism of resistance to heavy metals. A second aim was to define the biological costs of tolerance to heavy metals in *An. gambiae s.s.* following generational selection by cadmium, copper or lead.

Materials and methods

Heavy metals

Cadmium, copper and lead, respectively, were used in the following forms: cadmium chloride ($CdCl_2$) 99.99% pure (Fisher Scientific LLC, Fair Lawn, NJ, U.S.A.); copper II nitrate hydrate [$Cu(NO_3)_2$ 2.5 H_2O] > 99% pure (Sigma-Aldrich Laborchemikalien GmbH, Seelze, Germany) and lead II nitrate [$Pb(NO_3)_2$] 99.5% pure analytical salts (Prolabo, Fontenay-sous-Bois, France).

Test insects

Anopheles gambiae s.s. mosquitoes were obtained from a colony kept by the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. This colony was originally collected from Mbita field station (00°01′30″ S, 34°00′47″), South Nyanza province, Kenya in December, 2000, where *An. gambiae s.s.* is abundant in nature. At the time of this work, the colony was in the 35th filial generation post-field sampling. To our knowledge, the mosquitoes had not been previously exposed to heavy metals.

Mosquito rearing

Standard procedures for rearing *Anopheles* mosquitoes were followed (Ford & Green, 1972). All life stages were reared in an insectary under controlled environmental conditions (28 ± 2°C, 75–80% RH and LD 12 : 12 h photoperiod) in the Animal Rearing and Quarantine Unit (ARQU) of ICIPE, Nairobi. From the day of emergence, adult mosquitoes were provided with cottonwool soaked in a 10% sugar solution. Female mosquitoes were blood-fed on anaesthetized mice. Larvae were fed pulverized Tetramin fish food (Tetra GmbH, Melle, Germany). Approval for feeding mosquitoes on mice was obtained from the Kenya National Ethical Review Board (protocol number KEMRI/RES/7/3/1) and the protocol reviewed by the KEMRI Animal Care and Use Committee (ACUC).

Generation of metal-tolerant Anopheles gambiae s.s. *strains*

Anopheles gambiae s.s. third-instar larvae were selected for heavy metal tolerance tests through F_1 to F_3 generations, in empirically determined, maximum acceptable toxicant concentrations (MATCs) of cadmium, copper or lead. Eggs (1500 per replicate) and subsequent emergent immature stages (larvae and pupae) were exposed to the metal solutions (separately for each metal) in three replicates. The larvae were normally propagated in 1500 mL of the respective metal solutions in polypropylene cylindrical pans with a radius and height of 10.5 cm and 24.1 cm, respectively. The MATCs were 0.36 µg/L, 1.86 µg/L and 4.39 µg/L for cadmium, copper and lead, respectively. Tolerance to cadmium, copper and lead in the first and third generations was monitored through LC_{50} (50% lethal concentration) acute toxicity testing (Chareonviriyaphap *et al*., 2002).Toxicity range tests (24 h) for cadmium, copper and lead were conducted on the first and third generations using third-instar *An. gambiae s.s.* larvae. After determining the lower and upper toxicity ranges of each metal, 24-h acute toxicity tests were conducted. Lower and upper ranges were concentrations that caused >10% or <96% mortality, respectively. Three replicates ($n = 25$ larvae per replicate) were exposed to five lead, cadmium or copper concentrations within the established toxicity response ranges (Finney, 1971) in 400 mL of distilled water in the polypropylene cylindrical pans.

Larval mortalities were evaluated 24 h post-exposure and LC_{50} determined by probit analysis. The larvae were not fed during the exposure period. The generation of LC_{50} values, and respective slopes for each metal-selected strain, are presented in Table 1. A control colony was reared simultaneously in a separate room and handled in the same manner through all manipulations, but was not exposed to heavy metals. Emergent adult survivors from each treatment replicate and generation were propagated separately. A total of 12 colonies were used for each of three generations; the 12 colonies included nine heavy metal-tolerant treatment strains and three control strains.

All the concentrations were validated by direct quantitative determination of cadmium, copper or lead separately in each exposure concentration and replicate using a 210VGP flame

Table 1. Concentrations (μg/mL) that cause 50% mortality (LC_{50}) of third-instar *Anopheles gambiae s.s.* larvae in a naïve population and following three successive generational exposures to heavy metals.

Strain	Generation	LC_{50}, μg/L	95% CI	Slope ($\beta \pm$ SE)	χ^2
Cadmium	F_0	6.07	4.80–7.77	2.81 ± 0.41	0.17
	F_3	4.45	3.29–5.89	2.65 ± 0.49	0.34
Copper	F_0	12.42	8.34–18.45	1.76 ± 0.29	1.52
	F_3	25.02	18.20–35.59	2.12 ± 0.33	0.31
Lead	F_0	493.32	245.22–1007.77	1.03 ± 0.18	2.60
	F_3	516.69	272.44–1072.20	1.00 ± 0.16	1.55

95% CI, 95% confidence interval (for respective median value); SE, standard error.

atomic absorption spectrophotometer (Buck Scientific, Inc., East Norwalk, CT, U.S.A.) according to the manufacturer's instructions. Quality control was achieved using certified reference sediment material for cadmium, copper and lead (IAEA 433) from the International Atomic Energy Agency (Wyse *et al*., 2004).

Effect of heavy metal selection on immature An. gambiae s.s. *survivorship*

Egg hatchability. Samples of eggs ($n = 1500$) were collected separately from the third generation of selection for each of the strains (i.e. three heavy metals and one control × three replicates of each). Each sample of eggs was placed in 1500 mL of chlorine-free distilled water and the proportions of eggs that hatched were counted under a dissecting microscope (Leica WILD M3Z) at 48 h post-exposure.

Survival rate of larvae to eclosion. All emergent larvae were normally reared in separate 1500-mL containers as described above (i.e. for each of the three heavy metals and one control × three replicates of each). The initial larvae density for each treatment and replicate was therefore determined by the number of eggs that had hatched in each sample of eggs. Larvae pupating each day were noted and the pupae were placed into jars within emergence cages, separated by day, treatment and replicate.

Proportion of pupae surviving to produce adults and ratio of male : female adults. The numbers and sexes of adults emerging from each jar were recorded daily until the last adult emerged, constituting 12 readings of emergence rates (i.e. three heavy metal solutions plus one control × three replicates of each).

Effect of heavy metal selections on An. gambiae s.s. *adult survivorship*

A sample of emergent adults from each replicate was selected for adult life studies (Reisen & Mahmood, 1980). After males and females had been kept together in a cage long enough for mating to have occurred, samples of 40 males and 40 females (aged <12 h) from the control and each of the metal-selected strains were placed in separate 4-L plastic containers in three replicates. Each sample of mosquitoes was supplied with cottonwool soaked in 10% sucrose. Anaesthetized mice (female only) were provided daily as a bloodmeal source. For oviposition, water in a plastic cup lined with filter paper (9 cm radius) was provided. The egg cups and sucrose-soaked cottonwool were changed daily. The eggs collected represented a contribution from 40 females. The mortality of both sexes was recorded daily until the last mosquito died. The three replicates of each metal and control treatment were reared separately throughout post-selection processes and separate growing pans and cages were used for each replicate line in all assessments.

Data analysis

Mortality rates for testing the toxicity of each metal were corrected by Abbott's formula (Busvine, 1971) and then transformed to probits (Finney, 1971) for linear regression analysis and the determination of LC_{50} values. Probit analysis software was used (Probit Version 1.5; U.S. Environmental Protection Agency, Cincinnati, OH, U.S.A.).

Horizontal life table analytical methods were applied to data for both immature and adult stages of various selection categories because the cohorts were from distinct lines and were followed consistently through time from egg to adult (Reisen & Mahmood, 1980).

Age-specific survivorship of adults (l_x) was determined as:

$$l_x = y_x / y_0 \quad (1)$$

where y_x = the number of males or females alive on each day x and y_0 is the original number in the sample, so that the proportion is 1 on day 0.

The age-specific life expectancy (e_x) was computed as:

$$e_x = T_x / l_x \quad (2)$$

where:

$$T_x = \sum_{x}^{W} L_x \quad (3)$$

and:

$$L_x = (l_x + l_{x+1})/2 \quad (4)$$

and w = the day the last individual died (i.e. e_1 = the adult life expectancy at emergence in days).

In order to transmit the malaria parasites *Plasmodium falciparum*, *Plasmodium malariae* or *Plasmodium vivax*, the anopheline vector must survive for ~ 8, 14 and 7 days, respectively, at temperatures and humidities similar to those applied in this study (Siddons, 1944).Assuming the infective meal is taken during the mosquito's second and third nights of adult life, the potential infective proportion of the population would consist of females aged ≥10 days. Mean life expectancy at 10 days (e_{10}) was, therefore, computed for the female control and metal-selected strains. The net reproductive rate per cohort, or the total number of living females produced per female (R_0), was established as:

$$R_0 = a\sum_{x=1}^{W} l_x m_x \tag{5}$$

where a = the mean proportion of females that survived from egg through adult emergence, and:

$$m_x = E_x p \tag{6}$$

where E_x is the mean number of larvae (i.e. hatched eggs) produced per female per age interval x, and p is the proportion of the offspring that were female.

In this study, the mean values of 'a' (proportions of females surviving to adulthood) for the control, cadmium-, copper- and lead-tolerant strains were 0.85, 0.16, 0.18 and 0.11, respectively, based on magnitude of female emergence and the observed sex ratio; and the mean values of 'p' (proportion of offspring that were female) were 0.51, 0.49, 0.51 and 0.49, respectively. The p values are based on the observed sex ratios of the emerging adults from the non-selected control strain and metal-selected strains.

The age of mean cohort reproduction in days (T_0) was established as:

$$T_0 = a\sum_{x}^{W} l_x m_x x / R_0 \tag{7}$$

starting at $x = 1$, the day of adult emergence.

The instantaneous rate of increase in females per female (r_m) was calculated using the Dobzhansky *et al*. (1964) modification of the original Euler–Lotka equation by the Newton–Raphason iteration method where:

$$l = a\sum_{x=1}^{W} l_x m_x e^{-r_m(x+D)} \tag{8}$$

in which e is the base of the natural logarithm and D is the duration in days from oviposition in the present generation to first oviposition in the offspring generation. D was considered to be the observed mean median emergence time for females plus the duration of the nulliparous period for that cohort. For the non-selected control, cadmium-, copper- and lead-selected strains, D ranges were 14.66–17.33, 16.33–17.23, 16.56–19.07 and 14.23–14.85 days, respectively.

The mean generation time in days (G) was computed as:

$$G = \ln R_0 / r_m \tag{9}$$

Because this value included D in the calculation, G was a realistic estimate of the time from mean oviposition in the present generation to mean oviposition in the offspring generation.

The instantaneous birth rate (b) was calculated as:

$$b = \ln\ (1 + \beta) \tag{10}$$

and the instantaneous death rate (d) as:

$$d = (b - r_m) \tag{11}$$

where:

$$1/\beta = \sum_{x=1}^{W} L_x e^{-r_m(x+1)} \text{ (Birch, 1948)} \tag{12}$$

Population doubling time in days (T_d) was calculated as:

$$T_d = \frac{\ln(2)}{r} \text{ (Elkinton, 1993)} \tag{13}$$

The effects of the metal selection on egg viability/hatchability, larval and pupal mortalities, and male and female emergence were evaluated by one-way analysis of variance (ANOVA) on the three replicate datasets, with the control and each of the metal treatments as factors. Means that were significantly different were identified using Tukey's HSD (honestly significant differences) post hoc analysis. Similarly, the effects of heavy metal selection on the sex ratios, fecundities, male and female mean life expectancies from emergence (e_1), net reproductive rates, mean life expectancies at 10 days (e_{10}), (R_0) mean cohort reproduction ages (T_0), instantaneous rates of increases (r_m), mean generation times (G), instantaneous birth rates (b), death rates (d), population doubling times (T_d), r_m/b and b/d among the treatments were also evaluated by one-way ANOVA on respective triplicate datasets, with the control and each of the metal treatments as factors. Means that were significantly different were identified using Tukey's HSD post hoc analysis. ANOVAs and Tukey's HSD post hoc analyses were conducted using SPSS Version 11.5 (SPSS, Inc., Chicago, IL, U.S.A.).

Results

Changes in tolerance with metal selection

Cadmium was found to be the most toxic of the metals, followed by copper. The LC_{50} values for copper or lead selection generally increased with generation selection, whereas values for cadmium selection reduced for unknown reasons (Table 1). Overall, there was an approximately 1.36-, 2.01- or 1.05-fold change in LC_{50} values following cadmium, copper or lead selection, respectively. Computed slopes of regression lines for each generation, an indicator of resistance vs. tolerance development, indicate that values for β did not vary significantly.

Effects of metal selection on An. gambiae s.s. *immature survivorship, adult emergence and fecundity*

Effects of metal selections on various immature developmental attributes are presented in Table 2. The mean percentage of eggs that hatched was significantly higher in the control strain (98.5%) than in the metal-selected strains (63.8–66.6%) ($F_{3,11} = 90.11$, $P < 0.001$). Similarly, the mean numbers of larvae that died were similar among the metal-selected strains (68.9–79.0%) and significantly higher than in the control strain (10.1%) ($F_{3,11} = 121.23$, $P < 0.001$). The proportion of larval survivors was significantly higher in the control (89.9%) than the metal-selected strains, particularly the lead-selected strain, which had significantly fewer survivors (21.0%) ($F_{3,11} = 583.86$, $P < 0.001$). The pattern of pupal survivorship was similar to that of larval survivorship, with the survival of pupae exposed to cadmium (19.8%) being significantly higher than that of pupae exposed to lead (14.1%) ($F_{3,11} = 5.23$, $P < 0.05$).

In addition, significantly more males (87.8%) ($F_{3,11} = 719.26$, $P < 0.001$) and females (84.7%) ($F_{3,11} = 758.91$, $P < 0.001$) emerged from the control than from any of the metal-selected strains (11.2–18.0%). The male : female sex ratios were also similar among the strains ($F_{3,11} = 3.09$, $P > 0.05$). Fecundity (i.e. the mean number of eggs per female per group) was significantly higher ($F_{3,11} = 52.24$, $P < 0.001$) in the control strain than in metal-selected strains by 2.4-, 1.7- and 2.1-fold in the cadmium-, copper- and lead-selected strains, respectively. Overall, general survivorship was significantly higher in control than in metal-selected populations.

Effect of metal selection on An. gambiae s.s. *adult survivorship and fitness*

The effects of metal tolerance on the biological fitness of *An. gambiae s.s.* adults are presented in Table 3. The copper-selected strain had a lower mean life expectancy (e_1) than the cadmium- or lead-selected strains. Female life expectancies at 10 days (e_{10}) were similar ($F_{3,11} = 3.45$, $P > 0.05$) between all treatments. Net reproductive rates (R_0) were significantly higher ($F_{3,11} = 8.17$, $P < 0.01$) in controls by 6-, 20- and 21-fold than in the cadmium-, copper- and lead-selected strains, respectively. Metal-selected strains had significantly lower natural rates of increase (r_m) ($F_{3,11} = 5.53$, $P < 0.05$), but longer population doubling times (T_d) ($F_{3,11} = 7.81$, $P < 0.01$) than the respective control strains. Additionally, instantaneous birth rates (b) were significantly lower ($F_{3,11} = 5.72$, $P < 0.05$) in copper- or lead-selected strains than in the respective control strains. R_m/b ($F_{3,11} = 12.61$, $P < 0.01$) and b/d ratios ($F_{3,11} = 8.28$, $P < 0.01$) were significantly higher in control than in metal-selected strains.

Discussion

This study demonstrates the potential of *An. gambiae s.s.* mosquitoes to withstand chronic exposure to heavy metals within the ranges occurring in metal-polluted natural habitats (Mireji *et al*., 2008). Our findings are in harmony with recent reports (Awolola *et al*., 2007; Djouaka *et al*., 2007) that indicate the inherent capacity of this vector to adapt genetically to chemically altered habitats encountered in urban environments. This phenomenon may account for recent evidence of rapid increases in population sizes of some disease vectors in urban cities [reviewed by Robert *et al*., (2003)]. The present results indicate a 23-fold lower tolerance to the metals in our colony relative to the conditions in nature (Mireji *et al*., 2008), which may be attributed to the relatively shorter selection period (three generations) compared with those in natural populations in polluted habitats. The present study also indicates that tolerance occurs at significant fitness costs to the species, which is reflected in reduced survivorship and fecundity, as previously suggested (Orr, 1998; Reed *et al*., 2003). How this would affect the ecological performance of the mosquito remains unknown and detailed observations of

Table 2. Mean (±SE) and percentage developmental attributes of metal-selected and non-selected control *Anopheles gambiae s.s.* strains.

		Control		Cadmium		Copper		Lead	
Aspect	Attribute	$\overline{x}$	%	$\overline{x}$	%	$\overline{x}$	%	$\overline{x}$	%
Egg	Viability*	1477.00 ± 8.50^{a}	98.5	997.33 ± 34.72^{b}	66.5	998.67 ± 18.98^{b}	66.6	957.00 ± 32.75^{b}	63.80
Larvae	Mortality	149.67 ± 2.67^{a}	10.1	687.00 ± 19.47^{b}	68.9	687.67 ± 20.67^{b}	68.9	756.00 ± 42.58^{b}	79.00
	Survivorship	1327.33 ± 6.06^{a}	89.9	310.33 ± 27.42^{b}	31.1	311.00 ± 29.72^{b}	31.1	201.00 ± 15.70^{c}	21.00
Pupae	Mortality	33.33 ± 2.03^{ab}	2.5	61.33 ± 9.17^{a}	19.8	46.00 ± 8.50^{ab}	14.8	28.33 ± 2.33^{b}	14.12
	Survivorship	1293.33 ± 4.33^{a}	97.5	248.67 ± 18.22^{b}	80.2	264.33 ± 21.94^{b}	85.2	172.33 ± 14.26^{c}	85.88
Adult male	Emergence*	658.33 ± 11.46^{a}	87.8	126.33 ± 8.29^{b}	16.8	128.67 ± 12.47^{b}	17.2	87.33 ± 7.62^{b}	11.64
Adult female	Emergence*	635.00 ± 11.14^{a}	84.7	121.67 ± 10.04^{b}	16.2	135.00 ± 9.54^{b}	18.0	84.33 ± 6.69^{b}	11.24
	Fecundity	129.48 ± 8.11^{a}	–	54.88 ± 0.76^{bc}	–	47.18 ± 6.27^{c}	–	61.69 ± 2.09^{b}	–
Sex ratio	(Male/total)	0.51 ± 0.01^{a}	–	0.51 ± 0.00^{a}	–	0.49 ± 0.01^{a}	–	0.51 ± 0.00^{a}	–

Different letters (superscripts) in the same row (treatments) denote mean differences that are significant at a P-value of 0.05 by Tukey's HSD multiple comparisons.

*Percentage in relation to total number of eggs exposed, assuming a 1 : 1 sex ratio.

Viability, number of eggs hatching from 1500 eggs exposed; Mortality, number of larvae or pupae that died in the larval or pupal stage, respectively; Survivorship, number of larvae or pupae that developed into the next stage of the lifecycle; Emergence, number of male or female adult mosquitoes that emerged from the 1500 eggs exposed; Fecundity, mean number of eggs laid per female per group.

Table 3. Adult life table characteristics of control and heavy metal-selected *Anopheles gambiae s.s.* strains.

Attribute	Control	Cadmium	Copper	Lead
e_1 Male	8.05 ± 0.98[a]	5.84 ± 0.53[a]	5.95 ± 0.94[a]	6.15 ± 0.79[a]
e_1 Female	6.99 ± 0.89[a]	5.45 ± 0.35[a]	4.66 ± 0.27[b]	5.02 ± 0.48[a]
e_{10}	5.43 ± 0.60[a]	3.42 ± 0.27[a]	3.20 ± 0.91[a]	3.61 ± 0.51[a]
R_0	26.75 ± 8.01[a]	4.62 ± 3.07[b]	1.36 ± 0.23[b]	1.29 ± 0.06[b]
T_0	7.72 ± 0.46[a]	8.00 ± 0.49[a]	6.51 ± 0.39[a]	6.99 ± 0.27[a]
r_m	0.19 ± 0.06[a]	0.05 ± 0.03[b]	0.04 ± 0.02[b]	0.01 ± 0.00[b]
G	21.58 ± 0.29[a]	23.79 ± 1.24[a]	18.53 ± 5.42[a]	23.10 ± 0.43[a]
b	0.24 ± 0.01[a]	0.16 ± 0.04[a]	0.15 ± 0.01[b]	0.12 ± 0.01[b]
d	0.10 ± 0.02[a]	0.11 ± 0.01[a]	0.13 ± 0.01[a]	0.11 ± 0.01[a]
T_d	4.90 ± 0.66[a]	28.83 ± 11.61[b]	36.80 ± 6.53[b]	67.52 ± 12.81[b]
r_m/b	0.60 ± 0.08[a]	0.25 ± 0.10[b]	0.09 ± 0.04[b]	0.09 ± 0.02[b]
b/*d*	2.68 ± 0.47[a]	1.40 ± 0.22[b]	1.10 ± 0.05[b]	1.10 ± 0.02[b]

Different letters (superscripts) denote mean differences that are significant at a *P*-value of 0.05 by Tukey's HSD multiple comparisons.
e_1, mean life expectancy from emergence in days; e_{10}, mean life expectancy in days at 10 days post-emergence; R_0, net reproductive rate in living female progeny per female per generation; T_0, age in days at mean cohort reproduction; r_m, instantaneous rate of increase in living females per female; G, mean generation time in days; *b*, instantaneous birth; *d*, death rate, assuming stable age distribution; T_d, population doubling time in days.

affected field populations over a longer time-span may be necessary to determine their actual ecological fitness status in nature. In our previous study in polluted habitats (Mireji *et al*., 2008), we found *An. gambiae s.s.* thriving in significantly higher concentrations of heavy metals than those used in the present study, indicating that following survival under initial exposures, the population must have undergone intensive selection and that the fitness acquired was passed on to subsequent generations.

Our results have other potential implications for the ecological performance of mosquitoes. Firstly, because of the relatively high net reproductive rate (>1) of mosquitoes, metal-selected strains may be highly successful at colonizing habitats (Elkinton, 1993), although depressed egg viability and reduced fecundity in the adult population may lead to substantially reduced population sizes in successive generations. *An. gambiae s.s.* is known to proliferate in different types of habitat in different seasons (Mbogo *et al*., 1995), some of which have recently been found to be contaminated with heavy metals (Mireji *et al*., 2008). It may thus be anticipated that the dominance of metal-selected vs. naïve populations would alternate seasonally in different habitats, depending on the levels of heavy metals. The development of effective molecular markers for the two types of populations, and complementary field and controlled laboratory studies may help shed some light on these issues.

The differences in the patterns of tolerance to different metal selections may also suggest different underlying biological regulatory processes for each metal, including their uptake (Buchwalter & Luoma, 2005). The metal may have been absorbed through larval permeable body surfaces (Rainbow, 2007), the gut (Wang, 2002) or both. Additionally, significant variation between the effects of the metals on survivorship and fecundity indicate major differences in their effective toxicities to mosquitoes (Hare, 1992); the rate of detoxification of the respective metals could be an important determinant (Rainbow, 2002; Marsden & Rainbow, 2004). The variation may also reflect differences in the 'carry over' or transmission of some of the metal molecules from mother to offspring and/or other indirect maternal effects. Detailed comparative studies on molecular and physiological processes associated with tolerance and fitness, and particularly those that underlie sequestration of the metals and their bio-availability and bio-magnifications, are needed to elucidate reasons for these differences.

In conclusion, although *An. gambiae s.s.* displays the potential to develop tolerance to increasing levels of heavy metal, it does so at significant biological cost, which can adversely affect the mosquito's ecological performance and fitness. Our study provides a starting point from which further detailed complementary field and controlled laboratory studies may elucidate the longterm ecological implications of our findings.

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References

Afrane, Y.A., Klinkenberg, E., Drechsel, P., Owusu-Daaku, K., Garms, R. & Kruppa, T. (2004) Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? *Acta Tropica*, **89**, 125–134.

Agnew, P., Berticat, C., Bedhomme, S., Sidobre, C. & Michalakis, Y. (2004) Parasitism increases and decreases the costs of insecticide resistance in mosquitoes. *Evolution*, **58**, 579–586.

Awolola, T.S., Oduola, A.O., Obansa, J.B., Chukwurar, N.J. & Unyimadu, J.P. (2007) *Anopheles gambiae s.s.* breeding in polluted water bodies in urban Lagos, southwestern Nigeria. *Journal of Vector Borne Diseases*, **44**, 241–244.

Birch, L.C. (1948) The intrinsic rate of natural increase of an insect population. *Journal of Animal Ecology*, **17**, 15–26.

Buchwalter, D.B. & Luoma, S. (2005) Differences in dissolved cadmium and zinc uptake among stream insects: mechanistic explanations. *Environmental Science and Technology*, **39**, 498–504.

Busvine, J.R. (1971) *A Critical Review of the Techniques for the Testing Insecticides*. Farnham Royal, Commonwealth Agricultural Bureaux, London.

Chareonviriyaphap, T., Rongnoparut, P. & Juntarumporn, P. (2002) Selection for pyrethroid resistance in a colony of *Anopheles minimus* species A, a malaria vector in Thailand. *Journal of Vector Ecology*, **27**, 222–229.

Djouaka, R.F., Bakare, A.A., Bankole, H.S. *et al*. (2007) Does the spillage of petroleum products in *Anopheles* breeding sites have an impact on the pyrethroid resistance? *Malaria Journal*, doi:10.1186/1475-2875-6-56.

Dobzhansky, T., Lewontin, R.C. & Pallovsky, O. (1964) The capacity for increase in chromosomally polymorphic and monomorphic populations of *Drosophila pseudoobscura*. *Heredity*, **19**, 597–614.

Elkinton, J.S. (1993) *Insect Population Ecology; An African Perspective*. ICIPE Science Press, Nairobi.

Finney, D.J. (1971) *Probit Analysis*. Cambridge University Press, London.

Ford, H.R. & Green, E. (1972) Laboratory rearing of *Anopheles albimanus*. *Mosquito News*, **32**, 509–513.

Hare, L. (1992) Aquatic insects and trace metals: Bio-availability, bio-accumulation and toxicity. *Critical Review of Toxicology*, **22**, 327–369.

Levin, B.R., Perrot, V. & Walker, N. (2000) Compensatory mutations, antibiotic resistance and the population genetics of adaptive evolution in bacteria. *Genetics*, **154**, 985–997.

Marsden, I.D. & Rainbow, P.S. (2004) Does the accumulation of trace metals in crustaceans affect their ecology—the amphipod example? *Journal of Experimental Marine Biology and Ecology*, **300**, 343–371.

Matthys, B., N'Goran, E.K., Kone, M. *et al*. (2006) Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Côte d'Ivoire. *Journal of Vector Ecology*, **31**, 319–333.

Mbogo, C.M., Snow, R.W., Khamala, C.P. *et al*. (1995) Relationships between *Plasmodium falciparum* transmission by vector populations and the incidence of severe disease at nine sites on the Kenyan coast. *American Journal of Tropical Medicine and Hygiene*, **52**, 201–206.

Mireji, P.O., Keating, J., Hassanali, A., Mbogo, C.M., Nyambaka, H., Kahindi, S. & Beier, J.C. (2008) Heavy metals in mosquito larval habitats in urban Kisumu and Malindi, Kenya, and their impact. *Ecotoxicology and Environmental Safety*, **70**, 147–153.

Orr, H.A. (1998) The population genetics of adaptation, the distribution of factors fixed during adaptive evolution. *Evolution*, **52**, 935–949.

Rainbow, P.S. (2002) Trace metal concentrations in aquatic invertebrates: why and so what? *Environmental Pollution*, **120**, 497–507.

Rainbow, P.S. (2007) Trace metal bioaccumulation: models, metabolic availability and toxicity. *Environment International*, **33**, 576–582.

Reed, D.H., Lowe, E.H., Briscoe, D.A. & Frankham, R. (2003) Fitness and adaptation in a novel environment; effect of inbreeding, prior environment, and lineage. *Evolution*, **57**, 1822–1828.

Reisen, W.K. & Mahmood, F. (1980) Horizontal life table characteristics of the malaria vectors *Anopheles culifacies* and *Anopheles stephensi* (Diptera, Culicidae). *Journal of Medical Entomology*, **17**, 211–217.

Robert, V., Awono-Ambene, H.P. & Thioulouse, J. (1998) Ecology of larval mosquito, with special reference to *Anopheles arabiensis* (Diptera: Culicidae) in market-garden wells in the urban area of Dakar, Senegal. *Journal of Medical Entomology*, **35**, 948–955.

Robert, V., Macintyre, K., Keating, J., Trape, J.F., Duchemin, J.B., Warren, M. & Beier, J.C. (2003) Malaria transmission in urban sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene*, **68**, 169–176.

Siddons, L.B. (1944) Observation of the influence of atmospheric temperature and humidity on the infectivity of *Anopheles culicifacies* Giles. *Journal of Malaria Institute of India*, **3**, 363–384.

Trape, J.F. & Zoulani, A. (1987) Malaria and urbanization in central Africa, the example of Brazzaville. Part II, Results of entomological surveys and epidemiological analysis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **81**, 10–18.

Wang, W.X. (2002) Interactions of trace metals and different marine food chains. *Marine Ecology Progress Series*, **243**, 295–309.

Wyse, E.J., Azemard, S. & de Mora S.J. (2004) Report on the worldwide intercomparison exercise for the determination of trace elements and methyl-mercury in marine sediment IAEA-462 433, p. 113. IAEA/AL/147, IAEA/MEL/75. International Atomic Energy Agency, Geneva.

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