SCIENTIFIC REPORTS

OPEN

Received: 20 May 2015
Accepted: 25 August 2015
Published: 05 October 2015

An *ace-1* gene duplication resorbs the fitness cost associated with resistance in *Anopheles gambiae*, the main malaria mosquito

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Widespread resistance to pyrethroids threatens malaria control in Africa. Consequently, several countries switched to carbamates and organophophates insecticides for indoor residual spraying. However, a mutation in the *ace-1* gene conferring resistance to these compounds (*ace-1*^{R} allele), is already present. Furthermore, a duplicated allele (*ace-1*^{D}) recently appeared; characterizing its selective advantage is mandatory to evaluate the threat. Our data revealed that a unique duplication event, pairing a susceptible and a resistant copy of the *ace-1* gene spread through West Africa. Further investigations revealed that, while *ace-1*^{D} confers less resistance than *ace-1*^{R}, the high fitness cost associated with *ace-1*^{R} is almost completely suppressed by the duplication for all traits studied. *ace-1* duplication thus represents a permanent heterozygote phenotype, selected, and thus spreading, due to the mosaic nature of mosquito control. It provides malaria mosquito with a new evolutionary path that could hamper resistance management.

Vec o -bo ne di ea e , among hich mala ia i _ eeminen , ca e a con ide able b den on h man _ o_ la ion [1]. In b-Saha an Af ica, *An. gambiae* i he majo mala ia ec o . Mala ia accine i ill nde e e imen a ion and acce o an i-mala ia d g emain di c l and e en i e, h mo i o ec o con ol i he onl a o dable mea e o gh mala ia[2,3]. Mo i o con ol o ld ide elie e en iall on he e of chemical n he ic in ec icide ha a ge an in ec ' i al f nc ion[4]. Onl fo cla e of con en ional in ec icide a e licen ed b he Wo ld Heal h O gani a ion (WHO): O ganochlo ine (OC), P e h oid (PYR), Ca bama e (CX) and O gano_ho _ha e (OP)[5]. Di ec con ol of *Anopheles* b eeding i e i all no _o ible, and he main o_ ion o block o ed ce mala ia an mi ion i o _ e en he ec o -ho con ac ing in ec icide- ea ed bed-ne (ITN) and indoo e id al ho e _a ing (IRS).

Un il ecen l , PYR e e he onl in ec icide a ho i ed fo ITN and he mo ed fo IRS[4,6 12]. e e ool ha e been ho n o e ien l _ o ec lne able _o_ la ion f om endemic co n ie [1,13]. Unfo na el , d e o la ge- cale and _ olonged ea men , a ell a mo i o o_ la ion la ge e ec- i e i e and hei ho life _an _e gene a ion, e i ance o PYR a a idl elec ed and i no ide _ ead in mo mala ia ec o f om b-Saha an Af ica[14,15]. Se e al ala ming die _ edic ed ha PYR e i ance ma con ib e o mala ia ec o con ol fail e[15 18].

In o de o _ e e ec o con ol e ec i ene , al e na i e ol ion o PYR a e gen l needed. Ho e e , i h he limi ed n mbe of in ec icide and none e ec ed in he nea f e[14], OP and CX e e gge ed a _o en ial al e na i e com o nd o con ol PYR- e i an _o_ la ion , ei he alone o in combina ion i h PYR [17,19 21]. e ha e indeed ho n a good e cac in ITN and IRS, i h

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high mo ali of PYR- e i an (*kdr*^{R}) *An. gambiae* in I o Coa and Benin[22 24]. Th , follo ing he Ame ican P e iden ' Mala ia Ini ia i e in collabo a ion i h he Na ional Mala ia Con ol P og am, e e al Af ican co n ie ecen l i ched a l o en i el f om PYR o CX (i.e. bendioca b) o OP (i.e. chlo ifo o i imi ho me h l) fo IRS[18,22,25,26].

Ho e e, a a ic la conce n fo he e of OP and CX i ha e i ance o he e in ec icide i al ead e en in ome *An. gambiae* b la ion f om We Af ica[27 30]. Al ho gh e i ance ha al o been ho n o e l f om o e e e ion of de o ca ion en me [31,32], highe e i ance le el a e d e o m a ion in he a ge of OP and CX , he ace lcholine e a e (AChE1) encoded b he *ace-1* gene: a ingle amino acid b i ion of gl cine b e ine a he b i ion 119 (G119S) e l ing in a majo con- fo ma ional change[33]. i *ace-1*^{R} e i an allele a o e inde enden l e e al ime in di inc mo i o ecie [34,35]. In *Culex pipiens* mo i oe , i en ail a la ge ne co fo e e al life hi o ai [36 38]. A imila ne co a ea o e i fo *An. gambiae* (a ingle d ho ed ha ae ca ing *ace-1*^{R} end e highe mo ali a e[39]). , hile e i an mo i oe i e in he e ence of in ec icide, he a e o com e ed b ce ible in ab ence of in ec icide, d e o hei lo e ne . i ne co i c cial fo e i ance managemen : in ab ence of OP and CX elec i e e , *ace-1*^{R} f e enc ho ld indeed dec ea e (he co lie he fa e [40]), allo ing in ec icide o a ion o mo aic a egie o main ain lo le el of in ec icide e i ance.

Wo ingl , a ne *ace-1* allele ha been fo nd in *An. gambiae* and *An. coluzzii* in e e al We Af ican co n ie (e.g. I o Coa and B kina Fa o)[41,42]. i allele, named *ace-1*^{D}, con i in a d lica ion of he *ace-1* gene, a ocia ing a ce ible and a e i an co obabl on he ame ch omo ome. Se e al imila d lica ed allele ha e been ob e ed in *Cx. pipiens*[43,44], he e he ha e been ho n o be elec ed beca e he ed ce he ne co a ocia ed i h he G119S m a ion[45 47]. A elec i e ad an age a al o ecen l de c ibed in *Drosophila melanogaster* i h he d lica ion of he e i ance a ge gene *Rdl*^{R} [48]. A imila elec i e ad an age of *An. gambiae ace-1*^{D} allele o ld facili a e i di ion in na al b la ion , he eb eading OP and CX lo -co e i ance and endange ing mala ia ec o con ol a egie . I i h c cial o e al a e he h ea of hi *ace-1* d lica ion b in e iga ing i im ac on he ne of *An. gambiae*, bo h in e ence and ab ence of in ec icide.

To do o, e con c ed a labo a o ain homo go fo he *ace-1*^{D} allele and ha ing a gene ic backg o nd imila o he efe ence ain Ki m P and Ace ki , e ec i el homo go fo he ingle-co ce ible *ace-1*^{S} and e i an *ace-1*^{R} allele , a manda o e o a oid an confo nding e ec d e o he e i ance mechani m o an o he m a ion . We anal ed he o gani a ion of he d lica ed *ace-1* gene b a c ogene ic a oach, and com ared he h ee ain e fo mance fo OP and CX e i ance le el , a ell a e e al life hi o ai . i d e ealed ha *ace-1*^{D} i indeed e ec ed o ead h ea ening he i ch o OP o CX fo mala ia mo i o con ol in co n ie i h PYR- e i an b la ion .

Results

Characterization of the Acerduplikis strain carrying *ace-1* gene duplication. We collec ed la - ae f om a ild b la ion of *An. gambiae* in Bag ida[49], a egion ec ed o con ain *ace-1*^{D} allele , beca e of a la ge a a en e ce of he e o go [RS] heno e a he *ace-1* loc [42]. A no en ma ic o molec la e i c en l a ailable fo de ec ing *ace-1* d lica ion, e ed he gene ic o ocol de elo ed b Labbe *et al.*[44] fo *Cx. pipiens* mo i oe , o iden if female ha bo ing *ace-1*^{D} allele (S Fig. 1). e *ace-1*^{D} allele i com b ed of ce ible, D(S), and e i an , D(R), co ie (ee nomencla e in Labbe *et al.*[43]). e e e e enced in 16 female (2241 b PCR f agmen f om e on 2 o e on 7), and all D(S) co ie e e fo nd iden ical, a e e all D(R) co ie . e D(R) co i ic l iden ical o he kno n *ace-1*^{R} allele[32] and di e f om he D(S) co b 24 m a ion (S Fig. 2). Bo h D(S) and D(R) co ie e e fo nd iden ical o he e ence of he *ace-1*^{D} allele e io l de ec ed in *An. gambiae* Tacie [41]. P ogenie of he 16 fo nding female e e mi ed o con c he Ace d liki ain.

e n mbe of *ace-1* gene co ie a e ima ed fo 20 Ace d liki and Ki m P mo i oe i h Real- ime an i a i e PCR. Di e ence in co n mbe among D/D and S/S geno e a e ed b com ing he follo ing linea model Cn = Geno + ε, i h C he co n mbe , G he geno e (S/S o D/D) of each indi id al and ε he e o a ame e (Ga ian di ib ion). I con med ha Ace d liki igni can l di la ice a m ch *ace-1* co ie (2.13 ± 0.27) a Ki m P (1.00 ± 0.05; LRT, $F = 329.9$, $\Delta df = 1$, $p < 0.001$, S Fig. 3).

A o e cence *in situ* h b idi a ion (FISH) a oach a ed o locali e he b i ion of he o *ace-1* D(S) and D(R) co ie of Ace d liki *ace-1*^{D} allele on he ch omo ome . T o o e cen obe e e ed, *ace-1* and AGAP001373 (obe 2), hich a e e a a ed b abo 500 kb on he 2R ch omo ome a m of *An. gambiae*. O e l ho ed a ingle ignal i h he *ace-1* obe, a he ame loca ion fo bo h Ki m P and Ace d liki ain (Fig. 1A,B). Ho e e , he ignal a b oade fo Ace d liki . When he o obe e e co-h b idi ed on Ace d liki , bl ene ch omo ome , e ob e ed o di e en ignal , he b oade co e onding o he *ace-1* obe and he hinne o obe 2 (Fig. 1C). i e l e idenced ha he o co ie of he *ace-1* d lica ed allele a e in andem and e a a ed b a di ance lo e han 500 kb.

Finall , ince e aimed a de e mining he im ac of *ace-1*^{D} allele on mo i o ne , e e fo med eigh cce i e backc o e o in og e hi allele in o he ce ible Ki m P efe ence ain gene ic backg o nd. Pol mo hic e ence ma ke ha di e en ia e Ki m P and Bag ida (a mi of

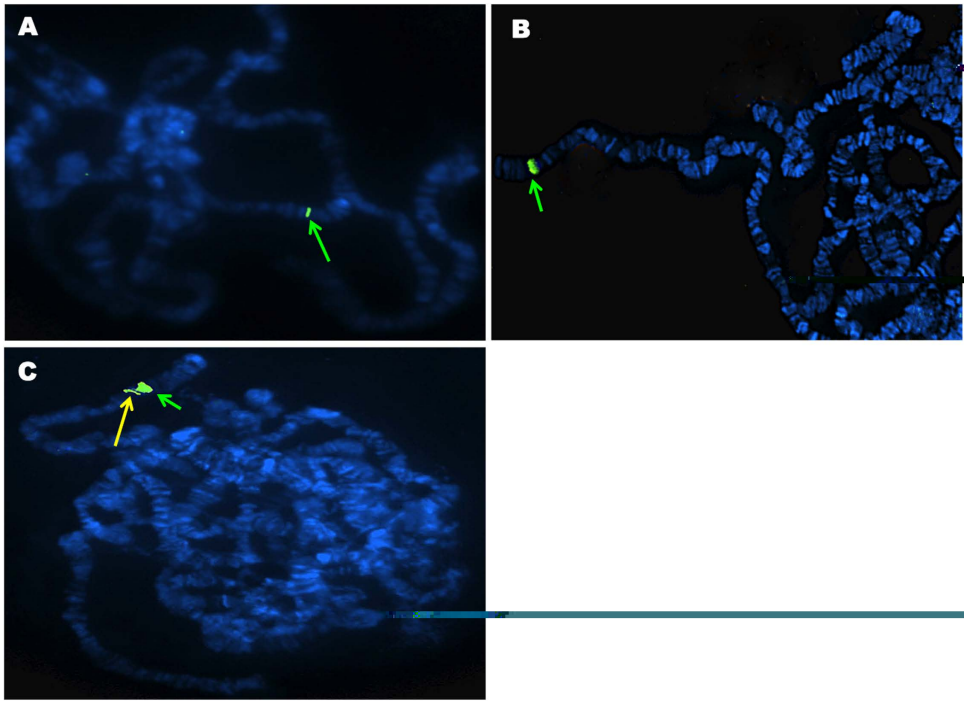


Figure 1. ***In situ*** **hybridization with Cy3 fluorescently labelled DNA probes performed on polytene chromosomes of** ***An. gambiae*** **strains.** G een and ello a o indica e *ace-1* and AGAP001373 _ obe e _ ec i el ; (**A**) *ace-1* _ obe h b idi ed on Ki m P ain; (**B**) *ace-1* _ obe h b idi ed on Ace d _ liki ain; (**C**) *ace-1* and AGAP001373 _ obe co-h b idi ed on Ace d _ liki ain.

10 indi id al f om he Bag ida eld _ la ion ed o e abli h Ace d _ liki) e e de elo_ ed on each ch omo ome (S _ lemen a Table 1). e Ace d _ liki ed ain ha ed all he Ki m P ma ke , ho ing ha he Ace d _ liki genomic backg o nd a la gel imila o ha of Ki m P. Al ho gh ecombina ion a o nd he *ace-1* gene i no o al, mo of he backg o nd e ec e e elimina ed, allo ing a _ e inen a e men of he d _ lica ion e ec on ne .

***ace-1*^{D} provides less resistance to carbamates and organophosphates insecticides than *ace-1*^{R}.** Bioa a e e ca ied o on la ae f om he h ee ain Ki m P (S/S), Ace ki (R/R) and Ace d _ liki (D/D) ain and f om hei F1 o _ ing (R/S, D/S and D/R geno e). One CX (bendioca b), h ee OP (chlo _ ifo me h l, feni o hion and dichlo o) and one PYR (e me h in) e e e ed. Fo all la al bioa a , mo ali in con ol e ne e e ceeded 5%. S a i ical anal e (chi- a e e be een ob e ed and e ec ed dead n mbe) indica ed good fo he log-do e-mo ali eg e ion (all p-*value* > 0.05, Table 1, S Fig. 4). Mo eo e , he ame ce_ ibili o _ e me h in a eco ded fo Ki m P, Ace ki (RR_{50} = 1, p > 0.05) and Ace d _ liki (RR_{50} = 1, p > 0.05) ho ing he ab ence of _ e h oid e i ance mechani m (Table 1). i la e l con med ha onl *ace-1* con ib ed o OP and CX e i ance in he e ed ain , e Ace d _ liki ain (D/D) di _ la ed a igni can l lo e e i ance le el o CX (bendioca b, RR_{50} = 3.14 *vs* 229.3, p < 0.001) and OP (chlo _ i ho -me h l, RR_{50} = 1.91 *vs* 9.03, p < 0.001; feni- o hion, RR_{50} = 6.56 *versus* 23.74, p < 0.001; dichlo o , RR_{50} = 8.78 *vs* 12.61, p < 0.001) han Ace ki (R/R; Table 1, S Fig. 4). While D/D indi id al di _ la ed a e i ance le el imila o he R/S he e o go e fo all he e ed OP (all p-*value* > 0.05), and a igni can l lo e e i ance le el fo he CX bendioca b (p < 0.001). Fo all he e ed in ec icide , D/S and D/R he e o go e di _ la ed, e _ ec i el , igni can l lo e and igni can l highe e i ance le el (all p- al e < 0.001) han D/D indi id al (Table 1), b D/R indi id al di _ la ed igni can l lo e e i ance le el han R/R indi id al (all p- al e < 0.001). F om he lea o he mo e i an , he geno _ e o de i h : SS < DS < DD ≈ RS < DR < RR.

***ace-1* duplication induces low, if any, fitness cost.** To mea e he ne co a ocia ed i h he di e en *ace-1* geno _ e , e e al life hi o ai e e com_ a ed in Ace ki (R/R), Ace d _ liki (D/D) and Ki m P (S/S).

Larval mortality and development time. - *Pre-imaginal mortality* a follo ed f om egg ha ching o ad l eme gence. e n mbe of dead la ae a each de elo_ men al age a eco ded, allo ing e ing

	Insecticides														
	Bendiocarb			Chlorpyrifos-methyl			Fenitrothion			Dichlorvos			Permethrin		
ace-1 genotypes	[a]LC_{50} (mg/L)	[b]RR_{50}	[c]Chi(p)	[a]LC_{50} (mg/L)	[b]RR_{50}	[c]Chi(p)	[a]LC_{50} (mg/L)	[b]RR_{50}	[c]Chi(p)	[a]LC_{50} (mg/L)	[b]RR_{50}	[c]Chi(p)	[a]LC_{50} (mg/L)	[b]RR_{50}	[c]Chi(p)
S/S (Ki m)	0.22	NA	0.99	0.004	NA	0.99	0.003	NA	0.99	0.008	NA	1	0.006	NA	0.99
R/R (Ace ki)	50.1	229.3	0.99	0.036	9.04	1	0.061	23.74	0.99	0.096	12.61	0.99	0.006	1	0.99
R/S	27.04	123.9	0.99	0.007	1.72	0.99	0.021	8.39	0.91	0.05	6.4	0.99	NA	NA	NA
D/S	0.28	1.29	1	0.006	1.56	0.99	0.016	6.28	0.99	0.04	5.4	0.99	NA	NA	NA
D/R	26.96	123.5	0.99	0.013	3.21	0.99	0.042	16.57	0.99	0.06	7.62	0.99	NA	NA	NA
D/D (Ace d liki)	0.68	3.14	1	0.007	1.91	0.99	0.022	8.78	0.99	0.05	6.56	1	0.006	1	0.99

Table 1. Dose-mortality responses to different insecticides observed in reference strains of *Anopheles gambiae* s. s. [a]LC_{50}: le hal concen a ion in millig am e li e ind cing a mo ali of 50%. [b]RR_{50}: e i ance a io a $LC_{50} = LC_{50}$(e i an ain)/LC_{50}(Ki m). [c]Chi(p): he p- al e of chi- a e e fo linea i y of he do e on e; p- al e > 0.05 indica e acce able (i.e. linea i y i no ejec ed).

fo di e ence be een ain in o e all mo ali a ell a in mo ali d namic . A Co o o - ional ha a d eg e ion model (Co model) a h com ed a : $S = G + \varepsilon$, i h S , he o o ion of dead la ae a each de elo men al age, G a h ee-le el fac o co e onding o he di e en geno e (S/S, D/D, R/R) and ε he e o a ame e , follo ing a binomial di ib ion o ake o Ta -di e ion in o acco n , if e en . Eme ging ad l e e cen o ed in he anal e .

e d lica ed D/D geno e di la ed a each la al age a igni can l lo e mo ali han he R/R geno e ($z = 3.6$, $p < 0.001$). Al ho gh i ended o be ligh l highe , D/D la al mo ali a each age a no igni can l di e en f om he ce ible S/S geno e ($z = 1.9$, $p = 0.06$; Fig. 2A). e o e - all la al mo ali ie of each geno e e e $m_{RR} = 0.71$ [0.60 0.79]; $m_{DD} = 0.43$ [0.32 0.52]; $m_{SS} = 0.29$ [0.20 0.38] (he 95% con dence in e al , o CI, a e gi en in he b acke).

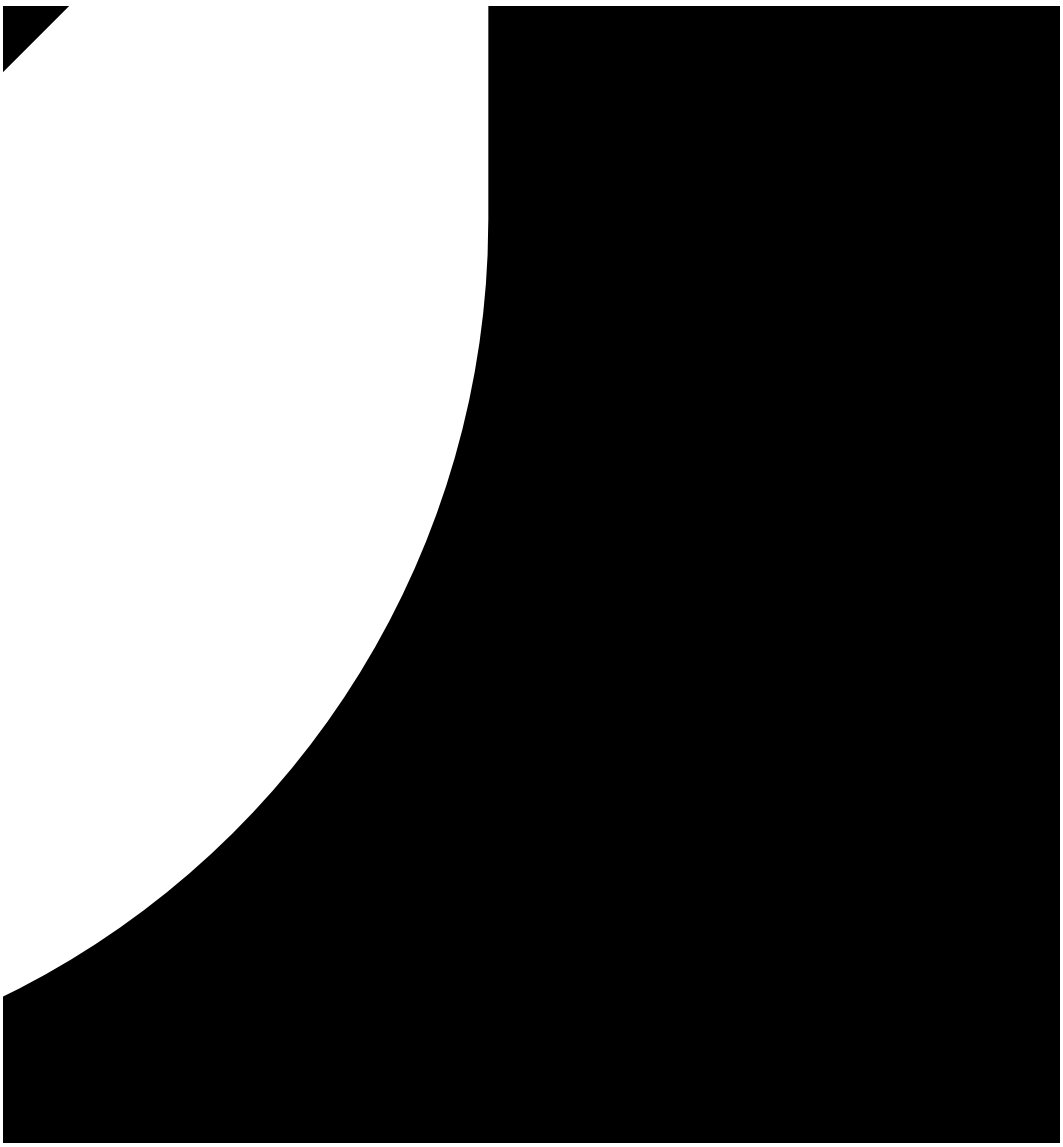
- Development time a eco ded a The n mbe of da nece a fo a -in a la a o each ad l hood (i.e. he ime n il eme gence). e e of each eme ging ad l a eco ded. Di e ence in de elo men ime be een geno e and/o e e e ed b com ing he follo ing Co model: $D = G + S + G.S + \varepsilon$, i h D , he n mbe of ad l ha eme ged a a gi en da , G a h ee-le el fac o (S/S, D/D and R/R), S a o-le el fac o (male o female), G .S he in e - ac ion be een he o fac o , and ε he e o a ame e (binomial di ib ion).

No in e ac ion be een e and geno e a de ec ed ($\chi^2 = 2.22$, $\Delta df = 2$, $p = 0.33$) allo ing d - ing he im ac of each fac o inde enden l . A e ec ed in *An. gambiae*[50], male eme ged igni can l ea lie han female (e ec i el : 8.5 ± 1.2 and 9.2 ± 1.3 da ; $\chi^2 = 8.9$, $\Delta df = 1$, $p < 0.01$). D/D indi id al de elo ed igni can l fa e han R/R indi id al (e ec i el , 8.7 ± 1.3 and 10.5 ± 0.84; $t = 7.85$, $df = 76.05$, $p < 0.001$). e mean de elo men ime a no igni can l di e en be een D/D and S/S indi id al (e ec i el , 8.7 ± 1.3 and 8.2 ± 0.7; S den e , $t = 0.83$, $df = 50.67$, $p = 0.41$; Fig. 2B). Ho e e , he Co model ho ed a la ge a iance in he de elo men ime of D/D han of S/S indi id- al (i.e. mo e ime be een he and he la ad l o eme ge; $z = 3.8$, $p < 0.001$; Fig. 2B).

Mating competition. Ma ing com e i ion ial e e e fo med be een ai of male of di e en gen- o e o com a e hei ca aci o in emina e ei he Ki m P (S/S) o Ace d liki (D/D) female . A gene ali ed linea model (GLM) a ed o com a e a e ni cce among com e ing male ai : $P = P + F + P.F + \varepsilon$, i h P he a e ni cce (n mbe of egg a i ed b a gi en male geno e), P a h ee-le el fac o co e onding o he ai of male geno e in he di e en ial (D/D *vs* S/S, D/D *vs* R/R, and S/S *vs* R/R), F a o-le el fac o co e onding o he female geno e (S/S o D/D), P .F he in e ac ion be een he e o fac o , and ε he e o a ame e (Binomial di ib ion).

e female geno e did no igni can l im ac he a e ni cce , ei he among he ial (P . F : $\chi^2 = 0.07$, $\Delta df = 2$, $p = 0.97$) o fo a gi en ial (F : $\chi^2 = 2.22$, $\Delta df = 1$, $p = 0.33$). Ho e e , he ai conf on ed in each ial did no fa e imila l (P : $\chi^2 = 11.25$, $\Delta df = 2$, $p < 0.01$). Bo h he D/D and he S/S male i ed mo e ogenie han R/R male (i.e. a e ni cce > 0.5): in DD *vs* RR ial, D/D a e ni cce a 0.68 ± 0.11 (> 0.5, Binomial e : $p < 0.001$), hile in SS *vs* RR ial, S/S a e ni cce a 0.68 ± 0.12 (> 0.5, Binomial e : $p < 0.001$). Pa e ni cce e of he D/D and S/S male e e no igni can l di e en , ei he hen conf on ed o R/R male ($\chi^2 = 0.02$, $df = 1$, $p = 0.89$) o o each o he : in DD *vs* SS ial, D/D a e ni cce a 0.48 ± 0.08 (no di e en f om 0.5, Binomial e : $p = 0.63$) (Fig. 2C).

Female fecundity and fertility. In o de o a e he in ence of he d lica ed allele on female e o- d c i e cce , fo female of each geno e (S/S, D/D and R/R) e e allo ed o la egg . e n mbe of female la ing egg , egg laid and la ae od ced e e eco ded.



O e all, he e_ od c i e cce (R) of R/R female a igni can l lo e (on a e age 21.5 ± 22 la ae _e female) han D/D o S/S female (e _ec i el , on a e age 33.1 ± 24 and 37.6 ± 33 la ae _e female; Fig. 2D) (GLM R c = Geno + ε (Ga ian di ib ion), $F = 7.04$, $\Delta df = 1$, $p < 0.01$). Mo eo e , he di e ence be een D/D and S/S female a no igni can ($F = 0.56$, $\Delta df = 1$, $p = 0.46$).

e ob e ed di e ence a e d e o he fac ha R/R female la fe e egg han he o he ; he n mbe of female la ing egg and he ha ching a e a e no igni can l di e en be een he h ee geno e . Fo a de ailed anal i ee S lemen a ma e ial S Fig. 3.

O e all, he o de of he geno e f om he le o he mo co l i : SS ≈ DD < RR.

Discussion

A mo e ad i o ge Af ican co n ie o i ch f om PYR o CX and OP fo mala ia ec o con ol, nde anding he al ead - ading e i ance o he e in ec icide i gen . e e en d con ib- e b cha ac e i ing he im ac of he *ace-1* gene d lica ion on *An. gambiae* ne . We con c ed a labo a o ain homo go fo he *ace-1*^{D} allele, ha ing a n clea backg o nd imila o he ce ible efe ence ain, Ki m P, o a oid an confo nding e ec . We hen de c ibed hi ne e i ance allele and mea ed i e fo mance in e ence o ab ence of in ec icide com a ed o ingle-co ce ible (*ace-1*^{S}) and e i an (*ace-1*^{R}) allele . O nding o ide cl e on ho *ace-1*^{D} ha a i en, b da ken he e ec i e of ing OP and CX a al e na i e o PYR ' l king inca aci a ion d e o i ing e i ance.

A new resistance allele at the *ace-1* locus. In *Cx. pipiens*, 13 di inc *ace-1* d lica ed allele ha e been iden i ed o fa [44,51,52], ome ime i h e e al d lica ed allele in a ame o la ion. Al ho gh no hing i kno n abo hei ch omo omal c e, he eem o ha e a i en f om e e al inde enden d lica ion e en [43].

e i a ion i i e di e en in *An. gambiae*, a onl one *ace-1*^{D} allele a ea o eg ega e in We Af ica: he ni e D(S) and D(R) e ence of he died allele he e and collected in Togo in 2012 a e iden ical o ho e e io l de c ibed in I o Co and B kina Fa o in 2006[41]. i ongl gge ha he oceed f om he ame d lica ion e en . e D(S) and D(R) co ie a e i e di e gen a he n cleo ide le el, b o d ho ha he la in clo e andem on ch omo ome 2R (Fig. 1). e e ob e a ion gge ha , among he di e en cena io gene a ing d lica ion [44], hi a ic la allele obabl e l f om an ne al c o ing-o e in a anda d he e o go e (i.e. R/S).

***ace-1* duplication sets a new evolutionary path for *Anopheles gambiae* resistance.** C en gen ing me hod fo he *ace-1* loc canno di c imina e *ace-1*^{D} ca ie f om a anda d he e o - go e. e efo e, *ace-1*^{D} f e enc in *An. gambiae* na al o la ion f om We Af ica ha been e i- ma ed f om he a a en e ce of [R/S] heno e ca ed b i e ence[42]. Ne e hele , e io e ima ion gge ed ha *ace-1*^{D} i i e f e en in hi egion[27,42,53]. O d o ide he cl e o nde and he ea on .

In ec icide e i ance da a ho ed ha he e i ance le el confe ed b he d lica ed allele *ace-1*^{D} i lo e han he one confe ed b *ace-1*^{R} (Table 1). I al o eem co ela ed o he e cen age of R co ie ca ied b mo i oe : fo all e ed in ec icide , di e en geno e i ance o de gene all a RR > DR > DD ≈ RS > DS > SS, imila l o ha a e io l de c ibed in *Cx. pipiens*[46]. A obable e lana ion co ld be he com e i ion e i ing be een AChE1S and AChE1R en me in he na e, a inc ea ing he n mbe of S co ie ill ed ce AChE1R en me andoml icked f om a ool, he eb dec ea ing he e i ance le el[45]. A e i ance ad an age (bo h in en i and eci ci) of *ace-1*^{D} o e *ace-1*^{R} i h clea l led o .

An. gambiae majo life hi o ai anal i (e-imaginal mo ali , la al de elo men ime, ma - ing com e i ion, and female fe ili) ho ed ha a high ne co i a ocia ed i h he e i an R/R geno e (Fig. 2A D), con ming he ole e io d a ailable[39]. Mo eo e , a an ici a ed[54], hi co i imila o he one a ocia ed i h he ame G119S m a ion in *Cx. pipiens* fo e e al life-hi o ai in eld and labo a o die [36,37,45,46]: fo in ance, in hi d he e-imaginal mo ali fo R/R homo go e i inc ea ed com a ed o he S/S 2.57 ime in *An. gambiae*, e 2.43 ime in *Cx pipiens*, (= 0.97, $p = 0.33$;[46]). e ne co a ocia ed i h *ace-1*^{R} a e io l a ib ed o he ed c ion of in en i i e ace lcholine e a e (AChE1R) ac i i b mo e han 60% com a ed o he ce ible one (AChE1S)[55], a magni de imila in bo h mo i o ecie [54].

While he e i ence of ch co fo *ace-1*^{R} com fo ho e of con olling hi e i ance allele, o e l a e g imme ega ding he d lica ed allele. e D/D geno e indeed a ea le co l han he R/R geno e, o he oin ha no igni can di e ence a ob e ed be een D/D and S/S geno- e on he fo life hi o ai mea ed (Fig. 2). e D/D geno e e fo mance e e ne e hele al a ligh l lo e han S/S one , hich co ld gge a lo ne co . A e io l o ed fo *Cx. pipiens*[46], he dec ea ed ne co in D/D indi id al com a ed o R/R co ld e l f om he ed c ion of he co l AChE1R ela i e an i (a he d lica ed allele od ce bo h AChE1R and AChE1S en me). To f he he com a i on be een he o mo i o ecie , he ne of *An. gambiae ace-1*^{D} allele eem o be a lea imila o ha of he e d lica ed allele anal ed o fa in *Cx. pipiens*, *ace-1*^{D1} [45,46]. Fo in ance, com a ed o R/R homo go e , D_1/D_1 e-imaginal mo ali i dec ea ed 1.3 ime in *Cx. pipiens*[46], e 1.7 ime fo D/D in *An. gambiae* ($z = -1.89$, $p = 0.06$).

A o i ome ob e a ion i ha he *Cx pipiens ace-1*^{D1} allele o all e laced he local *ace-1*^{R} in Ma ini e[56]. Simila l , *ace-1*^{D} a ea o be eading in We Af ica. O d e eal ha i highe ne doe no e l f om a highe e i ance le el b f om a dec ea ed co , o a he f om a ne e i- lib i m be een e i ance and co , o iding *An. gambiae* mo i oe i h a ne e ol iona a h.

***ace-1*^{D} is bad news for malaria vector control in areas with high PYR resistance.** Acco ding o he e l of o d , i i ob io ha he elec i e e in en i , in hi ca e he an i of in ec icide ed, ill be c cial o de e mine hich of *ace-1*^{R} o *ace-1*^{D} ill e ail in ea ed a ea ? in

highl ea ed a ea , *ace-1*^{R} ho ld be fa o ed d e o i highe e i ance le el, hile *ace-1*^{D} ill be e in le ea ed a ea , hank o i lo e co .

Ho e e , he he e ogenei of in ec icide age ac ice co ld ac all be de e minan on a la ge geog a hic cale and e ain he elec ion of he d lica ed allele in na al o la ion . D e o he mo aic na e of mo i o con ol and mo i o, hig a ion abili , a ame indi id al ma e e ience bo h ea ed and n ea ed a ea d ing i life an. i co ld fa o he elec ion of a mo e balanced, gene ali , heno ic o im m: a he e o go e indi id al (R/S) o ld i e be e han R/R indi id al in ab ence of in ec icide (lo e ne co), b al o be e han S/S indi id al in ea ed a ea . S ch he e o go e ad an age o e o con a ed en i onmen i called ma ginal o e dominance[57]. Ho e e , he e o go e canno become ed in a la ion, a he eg ega ion b den lead o he lo of he ad an age of ha ing bo h AChE1S and AChE1R en me in half of hei ogen . Haldane[58] o b ed ha he e i ence of o f nc ionall di e gen allele leading o o e dominance o ld omo e he eme gence and elec ion of a d lica ion ca ing bo h co le b c ea ing e manen he e o go e . Indi id al ca ing hi d lica ed allele o ld kee hei ad an age ac o gene a ion and ma in ade na al la ion . e *ace-1*^{D} allele in *An. gambiae* a ea a a e fec e am le of elec ion fo e - manen he e o go i : o d ho ha a D/D geno e e l in a heno e imila o a and- a d he e o go e R/S, b i ho he eg ega ion b den. Mo eo e , i di ib ion in We Af ican na al la ion bin o he c cial ole of he in ec icide age ac ice (and hei he e ogenei) in he elec ion of hi mo e gene ali allele. In B kina Fa o and I o Coa , CX and OP elec i e e e a ea mode a e b e a i e, a he *ace-1*^{D} allele i almo ed and *ace-1*^{R} f e enc i e lo [23,27,28,53,59]. In con a , bo h *ace-1*^{R} and *ace-1*^{D} f e encie a e ill lo in Benin, hich ce ainl e ec a lo elec i e e e[42,60].

Ho e e , hile in i ing f om a f ndamen al biolog bin of ie , he ead of *ace-1*^{D} in na al la ion co ld e e en a e io h ea fo e i ance managemen a egie . Indeed, ch a lo -co e i ance allele ill be mo e di c l o oo o ing cla ical a egie ba ed on in ec icide al e na ion. Mo eo e , he c en l -de lo ed Ame ican P e iden' Mala ia Ini ia i e in collabo a ion i h he Na ional Mala ia Con ol P og am co ld a o *ace-1* d lica ion ead if he in ec icide e - e i oo lo o he co e age oo he e ogeneo . i o ld elec fo e i ance o he ne IRS and ITN, and co ld be i e di a o . In high PYR e i ance a ea , *ace-1*^{D} o ld ead in la ion i h high f e encie of he *kdr* allele; e e io die ho ed ha *kdr* and *ace-1*^{R} (*ace-1*^{D}-kd in e ac ion ha no been in e iga ed o fa ac in ne g fo bo h e i ance le el and ne co [61,62]. F he mo e, a ecen d ho ed ha *ace-1* d lica ion a ocia ed i h en me de o ca ion eem o confe a e high bendio ca b e i ance o *An. gambiae* f om I o Coa [31]. I co ld h be ea ona- ble o ake befo ehand he ime o in e iga e mo e ho o ghl he o en ial im ac of a hi o CX and OP fo mala ia con ol, a ic la l in egion he e e i ance o he e in ec icide i al ead e en and he e *ace-1*^{D} i eading.

Methods

Mosquito strains and collection. Mo i o ain : o labo a o ain of *An. gambiae* e e ed in hi d : Ki m P and Ace ki . Ki m P ain a de i ed f om he efe ence ain Ki m - ce ible o all in ec icide [63]. A Ki m a he e ogeneo fo o ce ible allele a he *ace-1* loc , e i ola ed a ne ain, Ki m P, homo go fo a ingle ce ible allele (*ace-1*^{S} allele o S). Ace ki i a ain homo go fo he G119S m a ion in *ace-1* gene (*ace-1*^{R} allele o R), and e i an o bo h OP and CX in ec icide [30]. Bo h ain mo l ha e he ame Ki m gene ic backg o nd.

Mo i o collec ion: hi d in a la ae of *An. gambiae* f om Bag ida (6 09′47″N 1 19′50″E, Togo) e e elec ed i h o b a 1 mg/L (a concen a ion killing onl S/S indi id al) and e i an la ae e e ea ed n il ad l hood in he labo a o . A he ad l age, e ed mo hological e and molec- la anal i o iden if he membe of he *An. gambiae* com le e en [64–66].

Acerduplikis strain establishment. *Fixation protocol.* e a ion o ocol i ill a ed in S lemen a Fig e 1. I con i in fo cce i e e : (A) *Detection of females harboring the ace-1*^{D} *allele*: Female eme ged f om eld-collec ed la ae a e o b elec ion e e c o ed i h Ki m P S/S male and hen i ola ed o la egg . e o ing of each female a elec ed i h 1 mg/L o b . Mo he ha di la ed o ing i h no mo ali e e heno ed i h he *ace-1* RFLP-PCR e o iden if [RS] one[35]? All he e [RS] mo he co e onded ei he o D/R o D/D geno e , and h ha bo ed he d lica ed *ace-1*^{D} (D) allele. (B) *Elimination of the ace-1*^{R} *allele*: Once ad l, female e e c o ed i h Ki m P S/S male and e e allo ed o la egg indi id all . e e e hen c eened i h a PCR-RFLP e eci c of he D(S) co . Female iden i ed a D/S geno e e e e enced fo *ace-1* E 2-7 PCR f agmen (Tee belo). A all e ence e e fo nd iden ical, ogenie e e g o ed. (C) *Backcrosses on KisumuP*: e e female' ogenie e e ed fo i cce i e backc o e i h Ki m P male , in o de o homogeni e he gene ic backg o nd. (D) *Elimination of the KisumuP ace-1*^{S} *allele*: A e he la backc o , he ain a c o ed on i elf and elec ed i h 1 mg/L o b fo h ee gene a ion o inc ea e *ace-1*^{D} f e enc . P ogenie e e hen c eened i h a PCR e eci c of Ki m P. P ogenie in hich no Ki m P *ace-1*^{S} allele e e fo nd e e hen mi ed o con i e he Ace d liki ain, homo go fo he d lica ed allele *ace-1*^{D}, and ha ing a gene ic backg o nd la gel imila o Ki m P.

Genetic background characterization. A e eigh backc o e , mo of he Ace d liki ain a e ec ed o be in og e ed b he Ki m P gene ic backg o nd: a a 5% i k, all he genome e ce 30 cM a o nd he *ace-1* loc i e ec ed o ha e ecombined[36]. To check hi in og e ion, e de el- o ed a lea one molec la ma ke e *An. gambiae* ch omo ome ha a ol mo hic be een indi- id al f om he Ki m P ain and a mi of en indi id al f om he Bag ida eld o la ion ed o e abli h Ace d liki (S ! Table 1). e e ol mo hic ma ke e e hen e enced on DNA e ac ed f om a mi of abo 100 Ace d liki -in a la ae.

Specific molecular tests. All PCR e e e fo med i h 50 ng of genomic DNA in 40 μL nal nde he follo ing condi ion : 94 C fo 30 , annealing em e a e fo 30 , and 72 C fo 1 o 2 min fo a o al of 33 c cle (ime and annealing em e a e a e li ed in S ! Table 1).

- D(S) copy specific PCR-RFLP test. A PCR ing E on 3 ni di and AgE 4 e 2 ime am li e a 511 b f agmen f om all *An. gambiae ace-1* allele (S ! Tab. 1 and S ! Fig. 2). e e ic ion en me A aI c he *ace-1*^{S} and *ace-1*^{R} allele in o o f agmen (28 b and 483 b), and he D(S) co in o h ee f agmen (28 b 119 b and 363 b). 10 μL of he PCR od c e e dige ed i h 5 mi of en me fo o ho a 37 C.

KisumuP specific PCR test. A PCR ing Ki m di 2 and Ki m e 1 ime i eci c o he Ki m P *ace-1*^{S} allele; none of he o he *ace-1* allele e en a am li ed (S ! Fig. 2).

ace-1^D *sequencing.* Genomic DNA f om ingle mo i oe a am li ed ing he AgE 2di 1 and AgE 7 e 2 ime (2241 b PCR f agmen , f om e on 2 o e on 7 (S ! Fig. 2). PCR od c e e i ed ing he QIA ick Gel E ac ion Ki (QIAGEN). Fo he Ki m P and Ace ki ain , he i ed PCR od c a di ec l e enced. Fo Ace d liki , he i ed PCR od c a cloned ing he TOPO TA Cloning ki follo ing o he man fac e in T c ion (In i ogen Life Science Technologie), o e a a e he di e en d lica ed co ie , D(S) o D(R). e clone e e c eened fo he e ence of he G119S b i ion, and a lea i clone e e e enced fo each co . Se encing a cond c ed on an ABI P i m 310 e ence (BigD e Te mina o Ki , A lied Bio em). Fo e Ci , CA). Each clone a e enced ing he ime AgE 2di 1 and AgE 7 e 2, an in e nal ime d e o he f agmen leng h, AgIn di 1 (S ! Fig. 2). E on 2 o e on 7 e ence of he ce ible ingle-co allele f om Ki m P (Ag-*ace-1*^{S}), of he e i an ingle-co allele f om Ace Ki (Ag-*ace-1*^{R}) and of he ce ible (Ag-*ace-1*^{D}-S) and e i an (Ag-*ace-1*^{D}-R) co ie of he d lica ed allele e e de o i ed in GenBank (acce ion n mbe KM875634, KM875637, KM875635 and KM875636, e ec i el).

***ace-1* duplication mapping.** Onl he ain Ki m P and Ace d liki e e ed a hi age.

- Chromosomes preparation. O a ie e e lled o f om 4 da -old half-g a id female , 25 ho o blood-feeding, a Ch i o he ' S age III of de elo men [67], and e e ed in f e h Ca no ' a i e ol ion (3 ol me e hanol: 1 ol me glacial ace ic acid). O a ie e e ed fo 24 h a oom em e - a e and o ed a −20 C. Pol ene ch omo ome lide e a a ion a e fo med a de c ibed b Sha akho a *et al.*[68].

- Probes preparation. P obe 1 a eci c o he *ace-1* gene and obe 2 a eci c of he AGAP001373 gene, loca ed abo 500 kb f om *ace-1* on ch omo ome 2R in *An. gambiae* genome (h :// . ec o - ba e.o g/Ano hele gambiae). U ing Ki m P DNA, he obe 1 2241 b f agmen a am li ed i h AgE 2di 1 and AgE 7 e 2 ime and he obe 2 1861 b f agmen a am li ed i h Ag0.5MBdi 2 and Ag0.5MB e 2 ime (S ! Table 1). e e f agmen e e cloned i h TOPO TA Cloning ki follo ing he man fac e in c ion (In i ogen Life Science Technologie). DNA obe e e labelled e a a el i h DIG-Nick T an la ion Mi (Digo igenin-11-dUTP) acco ding o he man fac e P o ocol (Roche Diagno ic). H b idi a ion and de ec ion follo ed a e io l de c ibed oced e[69]. Fl o e cen ignal e e eco ded ing a Zei TA io ho mic o co e e i ed i h ha e-con a and o e cence image anal e (C o i ion 3.93.2). ee ol ene ch omo ome lide e e h b idi ed fo each ain and each obe hen co-h b idi ed fo Ace d liki ain i h obe 1 and obe 2.

Acerduplikis *ace-1* copy number quantification. e n mbe of *ace-1* gene co ie a e i- ma ed ela i el o a efe ence gene AGAP010592 = AgR 7 (fo nd in a ingle co in he Pe ain genome, Vec o Ba e h :// . ec o ba e.o g/) b Real- ime an i a i e PCR e fo med i h a LC480 Ligh C cle (Roche). T o PCR e e e fo med on each DNA, one eci c of *ace-1* loc (Agace1 idi 2 and Agace1 i e 2 ime) and he o he eci c of he efe ence gene (AgS7E 5 idi and AgS7E 5 i e ime) (S ! Table 1). 1 ng of each genomic DNA (no mali ed i h he Q bi 2.0 Fl o ome e -In i ogen) a mi ed i h 0.6 μM o 0.8 μM of *ace-1* o *Rps7* eci c ime e ec- i el and 3 μL of ma e mi (Ligh C cle 480 SYBR G een, Roche). PCR a e fo med i h a 95 C ac i a ion e fo 8 min follo ed b 45 c cle of 95 C fo 14 , 67 C fo 13 , and 72 C fo 19 . Each DNA em la e a anal ed in fo lica e fo bo h gene . e a io be een *ace-1* and *Rps7* a bi a concen a ion a de e mined i h he Ad anced Rela i e Q an i ca ion me hod of he Ligh C cle 480 o a e 1.5.0.

Bioassays with Carbamate and Organophosphate insecticides. Re i ance da a fo he h ee ain (Ki m P, Ace ki and Ace d liki) and hei F1 o ing (*ace-1* geno e R/S (Ace ki

Ki m P), D/S (Ace d liki Ki m P) and D/R (Ace d liki Ace ki)) e e com a ed. Fi e in ec- icide of echnical g ade ali e e ed one CX: bendioca b (99.5% e), h ee OP : chlo ifo me h l (99.9% e), feni o hion (95.2% e) and dichlo o (98.9% e), and one PYR: e me h in (98.3% e). In ec icide ol ion e e e a ed in 70% e hanol and o ed a 4 C in a da k oom o a oid ho ol i . A e of 25 la e hi d- and ea l fo h-in a la ae a inc ba ed in 99 ml of di illed a e in la ic c , o hich 1 ml of in ec icide ol ion a he e i ed concen a ion a added. Fo e lica e e e e fo med fo each concen a ion. Si o el e in ec icide concen a ion o iding a ange of mo ali f om 0 o 100% e e ed fo each in ec icide e ed. La al mo ali a eco ded a e a 24 ho e o e. Con ol bioa a e e e fo med b adding 1 ml of e hanol o 99 ml of di - illed a e . Tem e a e a main ained a 27 C ± 2 C d ing bioa a (em e a e mea ed ing Wa ane echnolog , Wa ane Sol ion, SAS, A ch, F ance).

Th e anal e of do e-mo ali e on e in bioa a e e e fo med ing he R o a e (.3.0.0). e R c i BioR a (6.1;[70]) a ed; i i f eel a ailable on he eb i e of he In i de Science de l'E ol ion de Mon ellie . i c i com e he do e of in ec icide killing 50% and 95% of he e ed b la ion o ain (Le hal Concen a ion 50 and 95, o LC_{50} and LC_{95}) and he a ocia ed con- dence in e al , and e fo he linea i of he do e-mo ali e on e (χ^2 e). Finall , i allo he com a i on of o o mo e ain o b la ion and calc la e he e i ance a io , i.e. RR_{50} o RR_{95} (=LC_{50} o LC_{95} of e ed b la ion/LC_{50} o LC_{95} of he efe ence ain, e !) and hei 95% con dence in e al .

Fitness cost parameters. *Larval mortality and development time.* To a e he de elo men ime and e-imaginal mo ali a ocia ed i h di e en *ace-1* allele , a a e e e fo med a de c ibed b Agne *et al.*[71]. Female o i b i ion a nch oni ed fo he h ee ain . A egg ha ching, 96 -in a la ae f om each ain e e indi id all an fe ed o *Drosophila* be fo ea ing in 1 ml of mine al a e a 2 g/L concen a ion of Te aMin o de ed h food (Te amin Bab Min, Te a Gmbh, Melle, Ge man). Food a o ided once, he da of e e imen . T be e e a anged on ack and main ained in in ec a condi ion (27 ± 2 C, 80 ± 2 h midi , 12 h: 12 h ligh :da k). e ack e e andoml mo ed e e da o ed ce b i ional e ec . Dead la ae o ae e e co n ed e e da o a e he mo ali a e a each de elo men age. Timing of ad l eme gence a al o eco ded.

Mating competition. Vi gin ad l (o-da old) ea ed nde labo a o anda d condi ion e e c o ed in cage (30 cm × 30 cm × 30 cm). T ial e e e fo med be een o male of each com e ing geno e (S/S *vs* R/R, S/S *vs* D/D o D/D *vs* R/R) laced in he e ence of ei he en S/S o en D/D female . Each com e i ion cage a e lica ed en ime . Mo i oe had acce *ad libitum* o a hone ol ion. A e h ee da , female e e blood-fed on abbi and allo ed o la egg indi id all . A e ha ching, each female ogen a elec ed i h an in ec icide do e ha allo a e ni a igna ion. When female e e S/S, a e ni in he S/S *vs* R/R and S/S *vs* D/D ial a a igned i h o b a 1 mg/L (hich kill onl S/S ogen); in he D/D *vs* R/R ial, a e ni a a igned i h bendioca b a 1 mg/L (hich kill D/S b no R/S ogen). When female e e D/D, a e ni in he S/S *vs* R/R and S/S *vs* D/D ial a a igned i h bendioca b a 1 mg/L, hile a e ni in he DD *vs* RR ial a a igned i h bendioca b a 5 mg/L (hich kill D/D b no D/R ogen). e a e ni cce of a gi en geno e a de ned in each e lica e of ial a he e cen age of egg- a i had ed.

Female fecundity and fertility. All ain e e ea ed nde he ame o en i onmen al condi ion and c o e e e e fo med be een 200 male and 200 female . A e a lea h ee da , female e e blood-fed and 40 g a id female f om each ain e e allo ed o o i b i indi id all in la ic c con aining 70 mL dechlo ina ed a e . ee da a e blood feeding, he n mbe of egg-la ing female and he amo n of egg e female e e eco ded. T o da a e , he n mbe of ha ching la ae e female a co n ed.

Statistical analyses. *ace-1* gene co n mbe a ia ion among S/S and D/D geno e a ana- l ed ing linea model . No mali of he model e id al and homo ceda ici e e checked ing Sha i o-Wilk and B e ch-Pagan e , e ec i el [70].

La al mo ali and de elo men ime e e anal ed ing Co o b ional ha a d eg e ion model[70].

Di e ence in a e ni cce be een ail e e e ed ing gene ali ed linea model (GLM), i h a binomial e o di ib ion. De a e f om he e ec ed o b ion of 0.5 i hin each ial (*i.e.* if he o male geno e di la he ame abili o fec nd female) a hen e ed ing e ac binomial e [70].

Di e ence among geno e in he a e of female la ing egg and in he ha ching a e e e e ed ing GLM i h binomial e o di ib ion . Di e ence among geno e in egg n mbe and la ae n mbe e female e e e ed ing GLM i h Ga ian e o di ib ion [70].

All com a ion e e e fo med ing he R f ee o a e (.3.1.1, h !// . - ojec .o g). Co ' model and GLM e e im li ed a follo : igni cance of he di e en e m a e ed a ing f om he highe -o de e m ing likelihood a io e (LRT). Non- igni can e m ($p > 0.05$) e e emo ed[72]. Fac o le el of ali a i e a iable ha e e no igni can l di e en e e g o ed (LRT[72]).

References

1. O gani a ion WH. Wo ld Mala ia e_ o 2013. Gene a: Wo ld Heal h O gani a ion; (ed^(ed) (2013).
2. Beie , J. C., ea ing, J., Gi h e, J. I., Macdonald, M. B., Im_ oin il, D. E. & No a , . J. In eg a ed ec o managemen fo mala ia con ol. *Malaria Journal* **7** S _ 1 1, S4 (2008).
3. Michala i , Y. & ena d, F. Mala ia: E ol ion in ec o con ol. *Nature* **462,** 298 300 (2009).
4. Whalon, M., Mo a-Sanche , D. & Holling o h, . *Analysis of global pesticide resistance in arthropods: Global pesticide resistance in arthropods*. CAB In e na ional, Camb idge, 192 (2008).
5. S oo_ , C. A., *et al.* En i onmen al fac o a ocia ed i h _a ial and em_ o al di ib ion of *Anopheles* (Di_ e a: C licidae) la ae in S ab mi, We Ja a, Indone ia. *Journal of Medical Entomology* **44,** 543 553 (2007).
6. G , W. & No a , . J. P edic ing he im_ ac of in ec icide- ea ed bed ne on mala ia an mi ion: he de il i in he de ail. *Malaria Journal* **8,** 256 (2009).
7. Lengele , C. In ec icide- ea ed bed ne and c ain fo _ e en ing mala ia. *Cochrane Database Syst Rev* **2,** CD000363 (2004).
8. Pl e , B., Tan e , F. C., Lengele , C. & Sha _ B. L. Indoo e id al _ a ing fo _ e en ing mala ia. *Cochrane Database Syst Rev* **4,** CD006657 (2010).
9. Ta en, W. nol BGJ. Mala ia ec o con ol: c en and f e a egie . *Trends in parasitology* **25,** 101 104 (2009).
10. an den Be g, H. *et al.* Global end in he e of in ec icide o con ol ec o -bo ne di ea e . *Environmental health perspectives* **120,** 577 582 (2012).
11. Zaim, M., Ai io, A. & Na a hima, N. Safe of _ e h oid- ea ed mo i o ne . *Medical and veterinary entomology* **14,** 1 5 (2000).
12. Zho , G., Gi he o, A. ., Mina a a, N. & Yan, G. Comm ni - ide bene of a ge ed indoo e id al _ a fo mala ia con ol in he We e n en a Highland. *Malaria Journal* **9,** 67 (2010).
13. Ei ele TP, L. . *et al.* E ec of ained in ec icide- ea ed bed ne e on all-ca e child mo ali in an a ea of in en e _ e ennial mala ia an mi ion in e e n en a. *The American journal of tropical medicine and hygiene* **73,** 149 156 (2005).
14. Na en, . In ec icide e i ance in di ea e ec o of _ blic heal h im_ o ance. *Pest Management Science* **63,** 628 633 (2007).
15. an on, H., N'G e an, ., Line , J., Moi o N., N ni, Z. & Co bel, V. P e h oid e i ance in Af ican *anopheline* mo i oe : ha a e he im_ lica ion fo mala ia con ol? *Trends Parasitol* **27,** 91 98 (2011).
16. N'G e an, ., Co bel, V., A ogbe o, M. & o land, M. ed ced e cac of in ec icide- ea ed ne and indoo e id al _ a ing fo mala ia con ol in _ e h oid e i ance a ea, Benin. *Emerg Infect Dis* **13,** 199 206 (2007).
17. Wo ld Heal h O gani a ion. *Global plan for insecticide resistance management in malaria vectors*. WHO _ e , Gene a, 24 (2012).
18. Sha _ B. L., idl, F. C., Go ende , D., lin i, J. & lein chmid I. Mala ia ec o con ol b indoo e id al in ec icide _ a ing on he o_ ical i land of Bio o, E a o ial G inea. *Malar J* **6,** 52 (2007).
19. G ille , P., N'G e an, ., Da ie , F., T ao e-Lami ana, M., Chand e, F. & Ca ne ale, P. Combined _ e h oid and ca bama e ' o-in-one' ea ed mo i o ne : eld e cac again _ e h oid- e i an *Anopheles gambiae* and *Culex quinquefasciatus*. *Med Vet Entomol* **15,** 105 112 (2001).
20. Ho ga d, J. M. *et al.* E cac of mo i o ne ea ed i h in ec icide mi e o mo aic again in ec icide e i an *Anopheles gambiae* and *Culex quinquefasciatus* (Di_ e a: C licidae) in Co e d'I oi e. *Bull Entomol Res* **93,** 491 498 (2003).
21. Zaim, M. & G ille , P. Al e na i e in ec icide : an gen need. *Trends Parasitol* **18,** 161 163 (2002).
22. A ogbe o, M., Padono , G. G., Ban ole, H. S., Ga a d, D. . & Gbedji i, G. L. D ama ic dec ea e in mala ia an mi ion a e la ge- cale indoo e id al _ a ing i h bendioca b in Benin, an a ea of high e i ance of *Anopheles gambiae* o _ e h oid . *The American journal of tropical medicine and hygiene* **85,** 586 593 (2011).
23. A idi, A. N. *et al.* E e imen al h e al a ion of bedne ea ed i h an o gano_ ho_ ha e (chlo _ ifo -me h l) o a _ e h oid (lambdac halo h in) alone and in combina ion again in ec icide- e i an *Anopheles gambiae* and *Culex quinquefasciatus* mo i oe . *Malar J* **4,** 25 (2005).
24. Fon aine E, P. J., Pa ne, D., P adhan, G. D., Jo hi, G. P., Pea on, J. A., ma i , M. . & amo Camacho, M. E. E al a ion of feni o hion fo he con ol of mala ia. *Bulletin of the World Health Organization* **56,** 445 (1978).
25. igo i, . *et al.* Indoo e id al _ a ing of in ec icide and mala ia mo bidi in a high an mi ion in en i a ea of Uganda. *PLoS One* **7,** e42857 (2012).
26. PMI. P e iden ' Mala ia Ini ia i e Eigh h Ann al e_ o . A ailable: h _ // . gh ingmala ia.go /doc /defa l - o ce/defa l - doc men -lib a /_ mi- e_ o /_ mi e_ o _ nal_ df (acce ed 19 Ma ch 2015), (2014).
27. Aho a Alo , L. P. *et al.* Di ib ion of *ace-1*^{R} and e i ance o ca bama e and o gano_ ho _ ha e in *Anopheles gambiae ss* _ o_ la ion f om Co e d'I oi e. *Malaria Journal* **9,** 1 7 (2010).
28. Dabi e, . . *et al.* Di ib ion of in en i i e ace lcholine e a e (ace-1) in *Anopheles gambiae* .l. _ o_ la ion f om B ina Fa o (We Af ica). *Trop Med Int Health* **14,** 396 403 (2009).
29. Djogbeno , L. *et al.* Iden i ca ion and Geog a hic Di ib ion of he ACE-1 M a ion in he Mala ia Vec o *Anopheles gambiae* in So h-We e n B ina Fa o, We Af ica. *Am J Trop Med Hyg* **78,** 298 302 (2008).
30. Djogbeno , L., Weill, M., Ho ga d, J. M., a mond, M., A ogbe o, M. & Chand e, F. Cha ac e i a ion of in en i i e ace lcholine e a e (ace-1) in *Anopheles gambiae* (Di_ e a: C licidae): e i ance le el and dominance. *J Med Entomol* **44,** 805 810 (2007).
31. Edi, C. V. *et al.* CYP6 P450 en me and ACE-1 d _ lica ion _ od ce e eme and m l i _ le in ec icide e i ance in he mala ia mo i o *Anopheles gambiae*. *PLoS genetics* **10,** e1004236 (2014).
32. Od ola, A. O. *et al.* E idence of ca bama e e i ance in ban_ o _ la ion of Ano_ hele gambiae mo i oe e i an o DDT and del ame h in in ec icide in Lago , So h-We e n Nige ia. *Parasites & Vectors* **5,** 1 8 (2012).
33. Weill, M. *et al.* Com a i e genomic : In ec icide e i ance in mo i o ec o . *Nature* **423,** 136 137 (2003).
34. Weill, M. *et al.* In ec icide e i ance: a ilen ba e _ edic ion. *Curr Biol* **14,** 552 553 (2004).
35. Weill, M. *et al.* e ni e m a ion in ace-1 gi ing high in ec icide e i ance i ea il de ec able in mo i o ec o . *Insect Molecular Biology* **13,** 1 7 (2004).
36. Be ica , C., Bo ien, G., a mond, M. & Che illon, C. In ec icide e i ance gene ind ce a ma ing com_ e i ion co in *Culex pipiens* mo i oe . *Genetical research* **79,** 41 47 (2002).
37. Bo g e , D., G illema d, T., Che illon, C. & a mond, M. Fi ne co of in ec icide e i ance in na al b eeding i e of he mo i o *Culex pipiens*. *Evolution* **58,** 128 135 (2004).
38. Leno mand, T. & a mond, M. e i ance managemen : he able one a eg . *Proc Roy Soc Lond* **265,** 1985 1990 (1998).
39. Djogbeno , L., Noel, V. & Agne , P. Co of in en i i e ace lcholine e a e in ec icide e i ance fo he mala ia ec o *Anopheles gambiae* homo go fo he G119S m a ion. *Malaria Journal* **9,** 12 (2010).
40. B o n, Z. S., Dic in on, Y. L. & ame , . A. In ec icide e i ance and mala ia ec o con ol: he im_ o ance of ne co mechani m in de e mining economicall o_ imal con ol ajec o ie . *Journal of economic entomology* **106,** 366 374 (2013).
41. Djogbeno , L. *et al.* E idence of in og e ion of he ace-1( ) m a ion and of he ace-1 d _ lica ion in We Af ican *Anopheles gambiae* . . *PLoS ONE* **3,** e2172 (2008).
42. Djogbeno , L., Labbe, P., Chand e, F., Pa e , N. & Weill, M. Ace-1 d _ lica ion in *Anopheles gambiae*: a challenge fo mala ia con ol. *Malar J* **8,** 70 (2009).

43. Bo g e , D., a mond, M., Bi e , J., Pa e , N. & A aga , M. D lica ion of he Ace.1 loc in *Culex pipiens* mo i oe f om he Ca ibbean. *Biochemical Genetics* **34,** 351 362 (1996).
44. Labbe, P. *et al.* Inde enden D lica ion of he Ace lcholine e a e Gene Confe ing In ec icide e i ance in he Mo i o *Culex pipiens*. *Mol Biol Evol* **24,** 1056 1067 (2007).
45. Labbe, P. *et al.* Fo ea of e a ic in ec icide e i ance e ol ion in he mo i o *Culex pipiens*. *PLoS Genet* **3,** e205 (2007).
46. Labbe, P., Mile i, P., Yeba ima, A., Pa e , N., Weill, M. & Leno mand, T. Gene-do age e ec on ne in ecen ada i e d lica ion : ace-1 in he mo i o *Culex pipiens*. *Evolution* **68,** 2092 2101 (2014).
47. Labbe, P., Sido , N., a mond, M. & Leno mand, T. e i ance Gene e lacemen in he Mo i o *Culex pipiens*: Fi ne E ima ion F om Long-Te m Cline Se ie . *Genetics* **182,** 303 312 (2009).
48. emnan , E. J. *et al.* Gene d lica ion in he majo in ec icide a ge i e, dl, in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences* **110,** 14705 14710 (2013).
49. An onio-N ondjio, C. *et al. Anopheles gambiae* di ib ion and in ec icide e i ance in he ci ie of Do ala and Yao nde (Came oon): in ence of b ag ic l e and ll ion. *Malar J* **10,** 154 (2011).
50. Clemen , A. Boo e ie ed: e Biolog of Mo i oe . Vol me 1. De elo men , N i ion and e od c ion. *American Journal of Tropical Medicine and Hygiene* **52,** 579 (1995).
51. Alo , H., Labbe, P., Pa e , N. & Weill, M. High incidence of ace-1 d lica ed ha lo e in e i an *Culex pipiens* mo i oe f om Alge ia. *Insect biochemistry and molecular biology* **41,** 29 35 (2011).
52. O a, M. A., i , Z. J., Labbe, P., Weill, M. & nio, . In ec icide e i ance o o gano ho ha e in *Culex pipiens* com le f om Lebanon. *Parasit Vectors* **5,** 1 6 (2012).
53. o , A. A., Alo , L. P. A., ab an, J.-P. ., N'G e an, . & Penne ie , C. e-Vi i ing In ec icide e i ance S a in *Anopheles gambiae* f om Co e d'I oi e: A Na ion-Wide Info ma i e S e , *PLoS One* **8,** e82387 (2013).
54. Alo , H., Djogbeno , L., Be ica , C., Chand e, F. & Weill, M. Com a i on of *Anopheles gambiae* and *Culex pipiens* ace choline e a e 1 biochemical o e ie . *Comp Biochem Physiol B Biochem Mol Biol* **150,** 271 277 (2008).
55. Bo g e , D., Pa e , N., Bi e , J. & a mond, M. De e mina ion of Ace.1 Geno e in Single Mo i oe : To a d an Ec menical Biochemical Te . *Pestic Biochem Physiol* **55,** 122 128 (1996).
56. Yeba ima, A., Ma ine, M., o ine, J., Y Tcha, M. M. & Pa e , N. E ol ion of e i ance nde in ec icide elec ion e e in *Culex pipiens quinquefasciatus* (Di e a, C licidae) f om ma ini e. *Journal of Medical Entomology* **41,** 718 725 (2004).
57. P o , T. S cien condi ion fo m l i le niche ol mo hi m. *American Naturalist* **102,** 493 496 (1968).
58. Haldane, J. B. S. *The biochemistry of genetics*. London, Geo ge Allen & Un in L d, (1954).
59. Edi, C. V., o do , B. G., Jone , C. M., Wee man, D. & an on, H. M l i le-in ec icide e i ance in *Anopheles gambiae* mo i oe , So he n Co e d'I oi e. *Emerg Infect Dis* **18,** 1508 1511 (2012).
60. Djogbeno , L., Pa e , N., A ogbe o, M., Weill, M. & Chand e, F. In ec icide e i ance in he *Anopheles gambiae* com le in Benin: a na ion ide e . *Medical and veterinary entomology* **25,** 256 267 (2011).
61. A ogba, B. S. *et al.* Pheno ic e ec of concomi an in en i i e ace lcholine e a e (ace-1) and noc do n e i ance (d) in *Anopheles gambiae*: a hind ance fo in ec icide e i ance manage men fo mala ia ec o con ol. *Parasites & Vectors* **7,** 548 (2014).
62. Be ica , C., Bonne , J., D chon, S., Agne , P., Weill, M. & Co bel, V. Co and bene of m l i le e i ance o in ec icide fo *Culex quinquefasciatus* mo i oe . *BMC Evol Biol* **8,** 104 (2008).
63. Sh e, G. A me hod of main aining colonie of Ea Af ican ain of Ano hele gambiae. *Annals of tropical medicine and parasitology* **50,** 92 (1956).
64. Fa ia, G., Lanf anco i, A., S ano , L., Siden iamo , I. & Lo i , C. Molec la cha ac e i a ion of ibo omal DNA ol mo hi m di c imina ing among ch omo omal fo m of *Anopheles gambiae* . . *Insect Molecular Biology* **10,** 19 23 (2001).
65. Gillie , M. & Coe ee, M. A S lemen o he *Anophelinae* of Af ica So h of he Saha a. *Publications of the South African Institute for Medical Research* **55,** 1 143 (1987).
66. Sco , J. A., B ogdon, W. G. & Collin , F. H. Iden i ca ion of ingle ecimen of he *Anopheles gambiae* com le b he ol me a e chain eac ion. *The American journal of tropical medicine and hygiene* **49,** 520 (1993).
67. Gillie , M. e d a ion of he gono o hic c cle in *Anopheles gambiae* and *Anopheles funestus*, i h a no e on he e cienc of hand ca ching. *East African medical journal* **30,** 129 (1953).
68. Sha a ho a, M. V., Xia, A., McAli e , S. I. & Sha a ho , J. V. A anda d c ogene ic ho oma fo he mo i o *Anopheles stephensi* (Di e a: C licidae): a lica ion fo h ical ma ing. *J Med Entomol* **43,** 861 866 (2006).
69. Ve ne , F. *et al.* A no el e de e mina ion em in a clo e ela i e of he ho e mo e. *Proceedings of the Royal Society B: Biological Sciences* **277,** 1049 1056 (2010).
70. Mile i Pa cal, L. P. Bio a : A Sc i fo Bioa a Anal e . 6.1. h // .i em ni -mon 2f / eche che/e i e / genomi edelada a ion/ e onnel/labbe- le ic , (acce ed 10 Oc obe 2014) (2013).
71. Agne , P., Be ica , C., Bedhomme, S., Sidob e, C. & Michala i , Y. Pa a i i m inc ea e and dec ea e he co of in ec icide e i ance in mo i oe . *Evolution* **58,** 579 586 (2004).
72. C a le , M. J. *The R Book*. John Wile & Son (2007).

Acknowledgments

We a e g a ef l o Nicole Pa e fo he hel f l commen on he man c i . i o k a f nded a Y b F ench ANR og am (ojec Al e NET SOC & ENV 2013-2015) and b Wellcome T (g an WT093755). A ogba S. Beno a o ed b a fello hi f om he IRD. Da a ed in hi o k e e od ced h o gh he echnical facili ie of he En i onmen al Genomic Pla fo m of he Labe Cen e Medi e aneen En i onnemen Biodi e i e (CeMEB): all e ence da a e e ob ained h o gh GenSe Pla fo m; FISH h b idi a ion a e fo med h o gh he E ol iona C ogenomic Pla efo m. Con ib ion n mbe 201 of he In i de Science de l'E ol ion de Mon ellie (UMR CNRS-IRD-UM 5554).

Author Contributions

B.S.A., L.S.D., P.L. and M.W. con ib ed o he conce ion and de ign of he e e imen ; B.S.A., A.B., J.P. and D.A. con ib ed o he da a ac i i ion; B.S.A., P.M., P.L. and M.W. con ib ed o he anal i of he da a; B.S.A., P.M. and M.W. d a ed he a icle and L.S.D., D.A., F.C., M.M. and P.L. e i ed i .

Additional Information

Supplementary information accom anie hi a e a h // .na e.com/ e

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Ogbaa, B. S. *et al.* An *ace-1* gene duplication resulting in reproductive compensation associated with insecticide resistance in *Anopheles gambiae*, the main malaria mosquito. *Sci. Rep.* **5**, 14529; doi: 10.1038/srep14529 (2015).