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Genetic pest management and the background genetics of release strains

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Genetic pest management (GPM) methods involve releasing modified versions of a pest species to mate with wild pests in the target area. Proposed for a wide range of applications in public health, agriculture and conservation, most progress has been made with pest insects. Offspring of the released modified insects and wild pests carry the modification—which might be transgenes, artificially introduced *Wolbachia* or genetic damage from radiation, for example—but they also carry a complete haploid genome from their laboratory-reared parent, as well as one from their wild parent. Unless these F_1 hybrids are completely unable to reproduce, further mating will lead to introgression of DNA sequences from the release strain into the wild population. We discuss issues around strain selection and the potential consequences of such introgression. We conclude that such introgression is probably harmless in almost all circumstances, and could, in theory, provide specific additional benefits to the release programme. We outline population monitoring approaches that could be used, going forward, to determine how background genetics may affect GPM.

This article is part of the theme issue 'Novel control strategies for mosquito-borne diseases'.

1. Introduction

Pest insects do enormous damage to human health (through the transmission of diseases such as dengue fever and malaria) and to agriculture (through damage to crops or livestock). Control methods, such as the use of insecticidal chemicals, are highly successful. However, their continued application may become restricted by concerns over environmental impact, and the evolution of chemical resistance. In the light of these drawbacks, there has been considerable investment in applying genetics-based approaches to pest control [1–3]. Genetic pest management (GPM) strategies aim to harness the natural mating systems of the pest in order to introduce into the pest population, traits that will sterilize, kill or otherwise modify the population. Here, the control agent is a version of the pest itself, laboratory-reared individuals with a heritable modification that desirably alters its properties are released into the wild. Such GPM has been proposed for a wide range of pest species, with a significant focus on insects, particularly mosquitoes [1].

While there are many inherent traits one might wish to change in a pest population, for GPM work has focused almost exclusively on two—fitness and vector competence. Introgression of traits such as sterility or lethality—reduced fitness traits—aims to reduce the numerical size of the target population, and so these are collectively described as 'population suppression' traits or methods. Reduced vector competence traits, relevant only to vector species, aim to reduce transmission of one or more pathogens with only

incidental effects on population size; these are 'population modification' methods, sometimes called 'population conversion' or 'population replacement' though the population is not typically actually replaced [1].

By far the most widely used GPM method to date has been the sterile-insect technique (SIT) and its variants, in which 'sterile' insects are released to mate with their wild pest conspecifics [4]. Introducing sterile insects, particularly sterile males, reduces the reproductive potential of the target population and with sustained releases can lead to the decline or even elimination of the target population. Sterility can be induced by irradiation [5–7], *Wolbachia*-induced cytoplasmic incompatibility [8–10], chemical treatment [11] or genetic engineering, for example, release of insects carrying a dominant lethal (RIDL) [12–15].

While much attention has focused on the novel genetic trait, the background genotype in which it is developed will contribute significantly to the performance of the released modified insects, for example, in terms of mating success. Considerably less attention has been paid to the capacity for hybridization and gene flow between the released modified insects and wild ones. 'Sterilization' of males is rarely 100% effective, for example, because of a need to balance sterility and negative effects on performance in selecting radiation dose, or incomplete penetrance of genetic sterility [16,17]. Other GPM methods require fertile matings to allow introgression of novel traits into target populations [14]; in both cases introgression of background genetic material is likely. This is a relevant question as introgression of novel genetic variation could affect the target population and perhaps even, in theory, result in an increase in fitness in the target population. With the rapid development of a variety of novel strategies, with varying propensities for gene flow, selection of background genotype should, therefore, be a significant decision for a developer, and correspondingly perhaps also for regulators and other stakeholders.

2. Population genetics of laboratory strains

Genetic differences have repeatedly been observed between laboratory colonies of mosquitoes and their source field populations, though their nature and magnitude vary substantially (reviewed in [18–20]). These laboratory insects differ from field ones owing to the combined effects of genetic drift, bottlenecking and selection [21]. Though the relative contribution of each has only rarely been studied [18], it is well recognized that laboratory strains have reduced fitness in the wild compared to their wild counterparts, leading to consistent concerns about the post-release performance of these strains for GPM [22].

Laboratory-reared wild-type strains typically have low effective population sizes, because founder population sizes are generally small, and lines are usually maintained at relatively low numbers after that. These small founding populations experience elevated levels of inbreeding and genetic drift relative to wild populations, leading to an accelerated loss of allelic diversity and heterozygosity [23–25]. Not surprisingly then, laboratory strains are consistently found to be less genetically variable than their field counterparts [26]. Small population sizes and low levels of genetic diversity present in laboratory strains result in reduced individual and population fitness through inbreeding depression and loss of adaptive potential. As such, theory predicts that, through neutral processes alone, laboratory populations should be less fit than their wild counterparts, especially in a wild setting [27]. In addition to these demographic processes, natural selection can also have large effects on individual fitness and population dynamics in laboratory populations. Laboratory environments are inherently artificial and colonized insect populations experience a substantially different set of selective pressures compared to those in natural populations (reviewed in [21]). Laboratory environments lack many selective pressures typical of wild populations, usually being very stable (controlled temperature, humidity, light : dark cycles and controlled rearing schedules) and benign (lacking many biotic and abiotic threats such as predators, disease, droughts or food scarcity) [23,28,29]. Simultaneously, laboratory rearing protocols involve artificial selection for a host of traits, including faster development to reproductive maturity and a front-loaded reproductive period [30–32], body size [24,33,34], longevity [24], courtship [35–38], blood-feeding behaviours [39–41], among others.

Genetic control strains probably experience even greater effects of bottlenecking and laboratory adaptation than laboratory-reared wild-type strains. As genetic modification is still highly inefficient, control strains must typically be constructed from those laboratory-adapted strains which are most productive in captivity. For example, after some success engineering *Aedes aegypti* [12,13], Oxitec—a company specializing in GPM technologies—attempted to develop similar strains of *Aedes albopictus*. With an eye to potential field use, they chose to start with a relatively recently colonized strain of *A. albopictus*, rather than a much older strain as had been used for *A. aegypti*. Successful engineering of *A. albopictus* was achieved [42,43] only after substantial improvements in strain productivity over 10 or so generations, attributed partly to improved methods but more to the further adaptation of the strain to laboratory conditions. Genetic control strains are also likely to experience a second bottleneck at their genesis, since they often originate from a single founder individual. This founder might carry a new transgene, chromosome translocation, or *Wolbachia* insertion. Recovering even the modest genetic diversity of a typical laboratory strain requires extensive backcrossing; there will be a further potential bottleneck if and when the strain is made homozygous, though this may not always be required. Inbreeding depression from such mating schemes may adversely affect the performance of the strain and potentially confound analyses of transgene-specific effects, e.g. fitness cost of the transgene [44]. On the other hand, one rarely considered aspect of genetic control strains is that they are often expanded to enormous population sizes for mass release. This may allow for some recovery of genetic variation through *de novo* mutations and increased recombination rates, although owing to the time scales involved it is unlikely that levels of genetic diversity in these populations will come close to that of wild populations. Further, rearing for mass-release programmes is likely to increase the strength and efficacy of adaptation to laboratory conditions, an issue that has long been of concern to SIT programmes [22].

3. Performance of laboratory strains in the wild

Empirical comparisons of laboratory and wild populations confirm the theoretical expectation that highly laboratory-adapted and inbred strains are unlikely to perform well in

the field, or at least less well than field populations [45–47]. There is, therefore, a trade-off between rearing efficiency, leading to lower unit costs (e.g. cost per million mosquitoes) and 'field-like' genetics leading to better per-mosquito performance [21]. Performance differences are not exclusively owing to background genetics; environmental conditions, rearing and distribution methods are also significant, as well as the effects of the novel trait, e.g. radiation-sterilization, *Wolbachia* or genetic engineering. Less well-understood differences such as the composition of the gut microbiota [48], which have been shown to be less diverse in most laboratory colonies of insects [49,50], may also be influential. There is some evidence that probiotic use is a potential route towards restoring population fitness for SIT [51,52].

SIT programmes have generally focused on improving rearing efficiency while trying to mitigate the inevitable reduction in performance. This has led to remarkably low-cost production, less than $1000 per million insects for a range of insect species (2008 USD, [53]). High-density, low-cost rearing is assumed to have a negative impact on field performance, but it is difficult to estimate the magnitude of this. For sterile males, male mating competitiveness is the key parameter of field performance. For example OX513A, an engineered strain of *A. aegypti* [12], was estimated to have a relative mating competitiveness of 0.031–0.14 varying across three open field trials in the Cayman Islands, Brazil and Panama [54–56]. This level of mating competitiveness, while low relative to wild males, is comparable to other large-scale, successful radiation-based SIT programmes; field competitiveness of sterile males was estimated at 0.1 for New World screwworm (*Cochliomyia hominivorax*) [6,57] and less than 0.01 for Mediterranean fruit fly (*Ceratitis capitata*) [58,59].

Any genetic and/or phenotypic differences between modified and wild mosquitoes raise the possibility of assortative mating. If wild females can recognize differences between wild and laboratory males, there may be strong selective pressure for females to avoid mating with released males. The possibility of 'behavioural resistance' through assortative mating was recognized from the early days of the SIT, with perhaps the best-documented instance coming from a melon fly programme in Okinawa [60,61]. Probably caused by a change in male courtship through mass-rearing, the selection pressure of prolonged releases of sterile males had induced a capacity in wild females to recognize these males and avoid mating [60]. Overall, this resistance was modest, and successfully managed simply by increasing the release rate of the sterile males. In practice, over the 60+ year history of the SIT, assortative mating has very rarely been observed [62].

Considerable efforts have been made to improve the performance of laboratory strains for GPM in the wild. Efforts to avoid the potential for behavioural resistance and optimizing male reproductive success include matching the background genetics of the release strain to that of the target population through backcrossing [24], and reducing the impacts of inbreeding, but whether this works in practice is difficult to determine. Oxitec's OX513A strain was originally constructed in a Rockefeller background, then backcrossed repeatedly into a laboratory strain originally collected in Tapachula, Mexico [54]. Rockefeller was colonized more than 100 years ago from mosquitoes collected in Cuba [63]. This strain was used successfully in the Cayman Islands, Brazil and Panama. Similarly, the classic Medfly (*C. capitata*) genetic sexing strains Vienna-7 and Vienna-8 and derivatives have been used successfully with a single composite genetic background in multiple territories [64]. These examples demonstrate that genetic backgrounds that are neither local nor recently colonized can be used successfully.

Strains can be periodically outcrossed to fresh material, e.g. recently wild-caught individuals. This may be more problematic for modified strains, depending on the nature of the modification. *Wolbachia* are maternally transmitted, meaning that new nuclear haplotypes can readily be introgressed *via* non-infected males. Such hybrids inherit half of their alleles from the mother and half from the father, so with each generation of backcrossing the residual contribution from the starting strain is expected to halve, on average, though that expectation may not be met if some alleles are under selection. In principle, this 'halving the residual background per generation' expectation could be improved by marker-selected breeding, though that is not commonly used for insects. There are also some special cases—in the *Wolbachia* example, the mitochondria are also maternally inherited, and therefore co-inherited with the *Wolbachia* so the mitochondrial genome cannot be exchanged by this route. For those transgenic strains that need to be made homozygous, changing the background genetics is considerably more onerous. The same process of repeated back crossing can be applied, but the resulting individuals are heterozygous for the transgene. The homozygous strain then needs to be rebuilt, and through this process will lose genetic diversity again. Similar issues may apply to strains made by classical genetics, for example, translocation-based genetic sexing strains. Furthermore, some parts of the genome may not be efficiently exchanged by this process. Sequences which are genetically linked to the novel trait will be retained through the outcrossing scheme, so-called 'hitch-hiking' effects.

Recognizing these problems, sophisticated schemes have been developed to preserve the genetic quality and diversity of strains reared in captivity, for example, the clean filter rearing system [65,66] and the use of multiple parallel sub-populations (genetically diverse laboratory strain, [67,68]). In a filter rearing system, a seed population is maintained at relatively low density, and potentially more naturalistic conditions. This population can be monitored as required for genetic integrity and quality. Eggs from this population are then taken for intensive rearing and expansion through a small number of generations to provide the release cohorts. The assumption is that genetic changes will have little time to accumulate between the seed population and release. Critically, material from the release population is never returned to the seed population—there is a conceptual one-way filter.

Though the novel trait(s) are not the main focus of this paper, it should be noted that background genetics can potentially affect the expression of novel traits. This has been clearly demonstrated for traits with intermediate penetrance, i.e. ones particularly sensitive to such variation, for example, for sterile-male systems [69] or some laboratory model gene drives [70], and is also known to have a significant effect on the spread of naturally occurring gene drives in some cases [71]. How relevant this is to the higher-penetrance traits likely to be used in the field is not clear and may vary between cases. Oxitec's OX513A was used successfully in the Cayman Islands, Brazil and Panama, and also tested in a range of other genetic backgrounds, without obvious

variation in effectiveness [54–56,72,73], on the other hand standing genetic variation or new mutation leading to heritable resistance to homing-based drives is a major concern for durable field use of such systems (e.g. [74] but see also [15]).

4. Population genetics and gene flow

GPM methods are likely to be imperfect, in that some individuals released from the laboratory into the wild do not fully express the intended trait. For radiation-based SIT, up to approximately 2% of males may be at least partially fertile (because radiation doses are calibrated to avoid the negative effect on insect performance of higher radiation doses), while offspring survival rates for OX513A RIDL mosquitoes have been estimated as 3–5% [12]. This raises the possibility of gene flow from laboratory to wild populations, which may have unintended consequences for individual fitness and population dynamics. Because of the reduced fitness of laboratory strains, levels of gene flow into wild populations are expected to be low. However, owing to the sheer scale of release programmes, at least some genetic introgression from the laboratory into wild strains is expected. Other genetic control methods, such as the use of female-specific lethal genes [13,75,76], or *Wolbachia*-based gene drives for population modification, can use lower release ratios but hybrids have much higher fertility, likely leading to more introgression of background genes. At the other end of the spectrum, highly invasive (low-threshold) gene drives may need only very low release ratios, so that introgression of background genetics is modest.

Gene flow into populations can have both positive and negative consequences for the receiving population. On the one hand, the introduction of novel genetic variation can counter the negative effects of inbreeding and genetic drift, and can provide novel variation upon which selection can act. On the other hand, gene flow can impede local adaptation and/or introduce deleterious variants. Theory suggests that the effects of gene flow on population fitness will depend on the rate of gene flow, the relative fitness of native and migrant individuals, and the effective population size [77]. However, very few studies have considered the dynamics of gene flow from large-scale release programmes.

In a recent study of a target mosquito population during and after a 27 month release trial of engineered *A. aegypti*, 10–60% of the target population were estimated to have at least some DNA derived from the release strain study, depending on the analysis used. There was, however, no evidence of gene flow into the nearest non-targeted population less than 3 km away [78]. This was interpreted as both undesirable and unexpected, but there seems little basis for such conclusions. Given the large release numbers associated with sterile-male methods, even the very modest level of survival by OX513A F_1 hybrid offspring would lead to observable genetic introgression, in the absence of any advantage to these 'laboratory-adapted' alleles, if these hybrids reproduced to any degree. The more pertinent question is whether introduced genetic variation persists at significant levels over extended periods, as has been observed between crops and wild relatives [79]. Using a temporal sampling approach, Evans *et al.* [78]. found consistent and marked decreases in levels of admixture with time since the release programme stopped. This strongly indicates selection against introgressed genotypes, although drift may have also played a role. This suggests, then, that selection against maladapted laboratory genotypes will act to remove most introgressed variation in a reasonably short amount of time.

Two caveats to the above arguments should be considered. Firstly, the Evans *et al*. [78] study is just one example, and in cases where selection against laboratory genotypes is weak, introgressed variation will persist for longer, and may become fixed owing to genetic drift. Secondly, and more importantly, quantifying introgression using approaches such as admixture analysis means that any inferences are based on genome-wide data, and do not account for localized introgression. Genome-wide studies of humans and other organisms have shown that highly localized regions of adaptive introgression can occur, can have large phenotypic effects, and be important for fitness [80]. Although it is hard to envisage laboratory strains containing alleles that would be adaptively introgressed into wild populations (see the section below), it is difficult to assess this using genome-wide approaches.

5. Fitness, vector competence and insecticide resistance

Evans *et al*. [78] caused immediate controversy asserting that hybridization would 'very likely' result in a more robust population 'due to hybrid vigour'—in apparent contradiction of their earlier conclusion that there was selection against introgressed alleles. Leaving aside the effect of the transgene, F_1 hybrids were likely to be fitter than the release strain owing to the combined effect of outbreeding and hybridizing with wild mosquitoes [18,81,82]. However, in comparison to the more genetically diverse target wild population, an influx of maladapted alleles from a highly inbred population is unlikely to have significantly increased levels of heterozygosity, removing the potential for heterosis and increased fitness.

Hybrid vigour, or heterosis, is one potential outcome from the introduction of new alleles into a population. Indeed, genetic rescue is a conservation strategy built around increasing the fitness of small, inbred populations by moving genes in from another population to increase genetic diversity [83], and this approach is rapidly emerging as a management tool to restore population health [84,85]. However, gene flow can also have negative effects on populations, for example, by breaking up local adaptation through the introduction of maladapted alleles [77,86]. In the case of the recent demise of the grey wolf population on Isle Royale, attempted genetic rescue by introducing migrants from the large mainland wolf population was blamed for producing a rapid population decline and bringing it to the brink of extinction [87]. As discussed above, mass-release strains are highly laboratory-adapted, having been through strong selection for rearing in an artificial habitat [21], making them poor candidates for producing a genetic rescue effect. Indeed, one of the major problems with mass-release methods is that the laboratory-reared insects perform rather poorly in the field.

Strong selective advantage at particular loci could allow for introgression at highly localized areas of the genome, which in turn could have consequences for population fitness and future pest control efforts. Insecticide resistance is a well-recognized issue in pest management, including GPM, and if a release strain were more resistant than the

wild population this would be under considerable selective pressure. In practice, standard laboratory strains are typically more susceptible to insecticides than field strains, since they were collected from the field many years ago and have been maintained without selection for resistance for tens or hundreds of generations. Nevertheless, this should be confirmed for actual release strains [88], as was the case for OX513A [89,90]. Interestingly, the lack of insecticide resistance was a problem for *A. aegypti*-wMel, the only mosquito gene drive system so far used in the field [91]. In Brazil, the *Wolbachia*-based gene drive failed to establish; this was attributed to use of a pyrethroid-susceptible gene drive strain [91]. A derivative strain with much higher pyrethroid resistance was developed and released to establish the gene drive. This may be a consideration for future genetic control strains, though different designs will differ in their sensitivity to this issue.

The vector competence (ability to transmit pathogens) of *A. aegypti* reflects virus genotype-by-mosquito genotype interactions, complicated by several environmental factors [92]. Our understanding of the genotype-to-phenotype outcome of genes affected by environmental interactions is limited, and the effect of introducing new alleles from laboratory strains may be difficult to predict, as acquired gut microbes, and plastic phenotypes such as body size are important interacting factors [93,94]. However, target populations are typically highly competent vectors—this is why they are target populations—and it is unlikely that laboratory strains are significantly better vectors. Evans *et al.* [78] found no evidence of a change in the vector competence of the hybrid mosquitoes, and to our knowledge no other studies have done so.

In addition to the potential negative impacts above—which need to be considered, though do not seem likely to be problematic in most cases—there is also the potential for the release strain to have more desirable genetic traits than the target population, such as reduced vector competence. This could be unrelated to the gene drive or genetic control trait and would mean that desirable alleles would enter the wild population with the potential for that population to correspondingly become somewhat more benign, though as previously discussed such effects may be highly localized and transient. A modelling-based analysis in the context of managing insecticide resistance found such introgression potentially very effective for slowing or even reversing the spread of insecticide resistance [95], an effect also observed in small glass-house studies [96]. This is a highly understudied area, where making predictions around the magnitude of the effect may be difficult, and depends on the amount of introgression, the fertility or otherwise of hybrids, and the type of genetic control strain.

If necessary, the unintended consequences of introgression could be reduced—but not eliminated—by repeatedly backcrossing the release strain to locally caught mosquitoes before releases start. This may be advantageous for a different reason, to improve the degree of mating between the released mosquitoes and the target population. On the other hand, more wild-like mosquitoes are likely to be harder to rear, being less laboratory-adapted, and consequently more expensive to produce. The cost of doing this for every target population is also likely to be prohibitive, and the limited data are mixed on whether such approaches are necessary for success. Oxitec's successful OX513A trials in the Cayman Islands and Brazil used a laboratory-adapted strain with primarily Mexican background [54]. On the other hand, as noted above, a *Wolbachia* gene drive strain failed to establish until matched to the local population genetics, with increased insecticide resistance considered to be the key trait [91].

6. Conclusion and recommendations

The selection of background strain will probably aim to balance several factors, including production traits and field performance. Existing precedent, primarily from sterile-insect methods, provides little support for the notion that strain background needs to be closely matched to either field strains in general or the target population in particular. Preliminary experiments may be able to identify major mating incompatibilities, as well as any adverse effects of the local genetic background on expression of the novel trait, and are, therefore, recommended at an early pre-release phase. Further research on the genetic and phenotypic changes associated with laboratory adaptation may enable us to better predict the potential and likelihood of any adaptive introgression into the wild. A better understanding of the fate of genes from mass-reared strains when released into the wild—perhaps through studying the large, long-term successful SIT programmes—seems prudent if we are to get a better understanding of the nature, extent and consequences of hybridization between laboratory and wild strains. A useful way forward in this respect may be to combine high-density sequencing with analyses designed to detect regions of adaptive introgression that are localized to specific parts of the genome [97].

There seem relatively few grounds for concern regarding introgression of laboratory genetic backgrounds into wild pest populations, though pre-release analysis of specific traits of concern (e.g. insecticide resistance, vector competence) would be wise, coupled with post-release monitoring as described. Indeed, introgression may in some instances become a desirable outcome of release programmes; for instance, we may wish to deliberately introgress background traits such as insecticide susceptibility into wild pest populations, in addition to any novel trait incorporated in the strain. Overall, more research into strain differences and its effects on field performance may help to optimize decisions about which strains to use in order to maximize the effectiveness of release programmes, while controlling the effects of gene flow and introgression.

Data accessibility. This article has no additional data.

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