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$$\frac{\kappa_1}{\kappa_0} = -\frac{\kappa_0}{\mu}\frac{4}{3}\left(\frac{\lambda+2\mu}{\lambda+\mu}\right)\left(\frac{1+i\omega\tau}{1+i\gamma\omega\tau}\right) \times\left[1-\left(\frac{1-\gamma^{-1}}{1+i\omega\tau}\right)\left(1-\frac{i(kl)^2}{\gamma\omega\tau}(1+i\gamma\omega\tau)\right)\right]^{-1}$$

where $\gamma = 1+\frac{a}{\pi c}\frac{\kappa_1}{\mu}\left(\frac{\lambda+2\mu}{\lambda+\mu}\right)$ and $\tau = \frac{\phi_m l^2\eta_l}{K_m\kappa_l}$;

λ and μ are the Lamé constants, ω and k are the angular frequency and wavenumber of the seismic wave, l is the interinclusion distance, a/c is the inclusion aspect ratio, κ_l and η_l are the bulk modulus and viscosity of the liquid, and ϕ_m and K_m are the porosity and permeability of the background matrix, respectively. Similarly, for the shear modulus, $\mu = \mu_0 + \phi\mu_1$, where

$$\frac{\mu_1}{\mu_0} = -\frac{2}{15}\left[\frac{8}{3}\left(\frac{\lambda+2\mu}{\lambda+\mu}\right)\left(\frac{1+i\omega\tau}{1+i\omega\gamma\tau}\right) + 16\left(\frac{\lambda+2\mu}{3\lambda+4\mu}\right)\left\{1+\frac{4a}{\pi c}\frac{i\omega\eta_l}{\mu}\left(\frac{\lambda+2\mu}{3\lambda+4\mu}\right)\right\}^{-1}\right]$$

We use the definition of attenuation as the ratio of imaginary (***Im***) to real (***Re***) parts of these expressions, and the corresponding *P*- and *S*-wave attenuation can then be defined as $Q_P^{-1} = \boldsymbol{Im}(\kappa+4\mu/3)/\mathrm{Re}(\kappa+4\mu/3)$ and $Q_S^{-1} = \boldsymbol{Im}(\mu)/\mathrm{Re}(\mu)$, respectively. Fixed parameters used in attenuation calculation (Fig. 4; Web fig. 3): $\lambda_0 = 941$ GPa, $\mu_0 = 636$ GPa, $\omega = 2\pi$, $kl = 10^{-6}/\phi$, $\phi_0 = 0.1$, $\kappa_f = 900$ GPa, and $K_m = 10^{-18}$ m^2.
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Insect Population Control Using a Dominant, Repressible, Lethal Genetic System

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A major modification to the sterile insect technique is described, in which transgenic insects homozygous for a dominant, repressible, female-specific lethal gene system are used. We demonstrate two methods that give the required genetic characteristics in an otherwise wild-type genetic background. The first system uses a sex-specific promoter or enhancer to drive the expression of a repressible transcription factor, which in turn controls the expression of a toxic gene product. The second system uses non–sex-specific expression of the repressible transcription factor to regulate a selectively lethal gene product. Both methods work efficiently in *Drosophila melanogaster*, and we expect these principles to be widely applicable to more economically important organisms.

The sterile insect technique (SIT) is a species-specific and environmentally nonpolluting method of insect control that relies on the mass rearing, sterilization, and release of large numbers of insects (*1*, *2*). Released sterile males mate with wild females, reducing their reproductive potential and, ultimately, if enough males are released for a sufficient time, totally eradicating the pest population. Successful, area-wide SIT programs have been conducted against the screwworm fly *Cochliomyia hominivorax* (*2*), the Mediterranean fruit fly (Medfly) *Ceratitis capitata* (*3*), and the tsetse fly (*Glossina* spp.) (*4*).

Mass-rearing facilities initially produce equal numbers of the two sexes, but females are generally separated and discarded before release. Sterilized females are not thought to help control efforts and may indeed be detrimental to them (*5*). Mechanical sex-separation methods using pupal mass, time of eclosion, and so forth rarely yield a true single-sex population. Various female-killing and sex-sorting genetic systems have been developed, known generically as genetic sexing mechanisms (GSMs). So far, all GSMs in factory production have used radiation-induced translocations to the Y chromosome as dominant selectable markers, complementing an X-linked or autosomal recessive trait such as pupal color, temperature-sensitive lethality, blindness, or insecticide resistance (*5–7*). These chromosome aberration–based systems tend to be unstable and reduce the fitness of the insects, making them less effective agents for SIT (*5*).

A better approach would be to use a transgene system to induce repressible female-specific lethality. This could be used simply as a GSM. In addition, these transgenics could be used in a control program without requiring sterilization by irradiation. We call this variant of SIT "release of insects carrying a dominant lethal" (RIDL), because the insects are not, strictly speaking, sterile. RIDL requires that a strain of the target organism carries a conditional, dominant, sex-specific lethal, where the permissive condition can be created in the laboratory or factory but will never be encountered by the wild population. An ideal example would be a chemical additive to the diet.

To demonstrate the feasibility of RIDL, we attempted to construct the system in *Drosophila melanogaster*. We used *Drosophila* transcriptional control elements to drive expression of the tetracycline-repressible transactivator fusion protein (tTa) (*8*). In the absence of tetracycline, tTa will drive expression of any gene controlled by the tetracycline-responsive element (tRe). We first expressed tTa under the control of the Yp3 fat-body enhancer (*9*). This drives expression in female larvae and adults, but not in males (*10, 11*). Because yolk proteins or vitellogenins are expressed in a similar pattern in most insects, we expect these promoters to be useful for RIDL in insects of economic importance. To test for female-specific tTa expression, we used a tRe-lacZ reporter (*12*).

Flies homozygous for stable insertions of Yp3-tTa or tRe-lacZ were crossed to each other. The resulting progeny were raised either on normal media or on media supplemented with tetracycline. Adult abdomens were dissected and stained for lacZ activity. Females raised on normal media showed strong staining of the fat body, whereas females raised on tetracycline, and all males, were negative (*13*).

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Table 1. Tetracycline-repressible lethality with tRe-Ras64B^{V12}. Males homozygous for tRe-Ras64B^{V12} and virgin females homozygous for Hsp26-tTa or Yp3-tTa were placed on media with or without tetracycline supplement. Their progeny were allowed to develop on this media. Adult F_1 survivors were counted. In the absence of tetracycline, no Hsp26-tTa/+; tRe-Ras64B^{V12}/+ survived. Male, but not female, Yp3-tTa/+; tRe-Ras64B^{V12}/+ survived. Males and females of both genotypes all survived on media supplemented with tetracycline. Data are the sum of at least four experiments with different insertion lines of the tRe and tTa constructs.

Tetracycline (μg/ml)	Hsp26-tTa		Yp3-tTa	
	Male	Female	Male	Female
0	0	0	350	0
0.1	210	235	352	322
1	274	199	376	342

Table 2. Selective elimination of females with sex-specific expression and/or sex-specific toxicity. Males and females homozygous for a tTa construct and a tRe construct (*18*) were maintained on media with the indicated tetracycline supplement and then transferred to normal (tetracycline-free) media. F_1 progeny were counted. This genetic sexing is efficient (no female progeny recovered) over a wide range of parental tetracycline concentrations. Yp3-tTa, female-specific tTa; tRe-Ras64B^{V12}, nonspecific tRe; Hsp26-tTa, nonspecific tTa; and tRe–msl-2^{NOPU}, female-specific tRe.

Tetracycline (μg/ml)	Yp3-tTa, tRe-Ras64B^{V12}		Hsp26-tTa, tRe–msl-2^{NOPU}		Yp3-tTa, tRe–msl-2^{NOPU}	
	Male	Female	Male	Female	Male	Female
0.1	1363	0	1523	0	1231	0
1	1360	0	1367	0	1209	0
5	1384	0	1478	0	1323	0
20	1366	0	1221	0	1168	0

The fat body is an essential organ whose functions include processing and storing nutrients and synthesizing components of the insect's immune system (*14, 15*). We therefore expected expression of a cytotoxic gene product in the Yp3 pattern to be lethal to females. We constructed tRe-Ras64B^{V12}, an activated Ras64B (*16*) under the control of tRe sequences (*9*). We crossed a constitutive tTa line [Hsp26-tTa (*12*)] to tRe-Ras64B^{V12}. Grown on media containing tetracycline, the progeny were viable and fertile. On normal media, no progeny survived (Table 1). A version of this system expressing tTa early in development only could be used as an alternative to radiation sterilization.

Flies homozygous for Yp3-tTa were crossed to flies homozygous for tRe-Ras64B^{V12} on media with and without tetracycline. In the presence of tetracycline, the resulting progeny had a normal sex ratio and showed no detrimental effects linked to the transgenes. On normal media, no female progeny survived (Table 1).

A stable population of flies was constructed, homozygous for Yp3-tTa and tRe-Ras64B^{V12} on a single chromosome. These flies were maintained on media containing tetracycline to inhibit the expression of Ras64B^{V12}. Populations of such flies were then transferred to media containing various concentrations of tetracycline. No female progeny were recovered from normal media, in comparison to >5000 male progeny (Table 2). These males were viable and fertile; when mated to nontransgenic females of several genotypes, they produced no female progeny. These flies therefore performed exactly as required for RIDL.

Female-specific lethality can also be achieved with a gene product that is toxic only to females. We used *msl-2*^{NOPU}, a mutant version of *male-specific lethal 2* (*msl-2*), a gene involved in dosage compensation (*9*, *17*). *msl-2*^{NOPU} activates the dosage compensation mechanism in both males and females. We investigated the consequences of expressing *msl-2*^{NOPU} in females, where the dosage compensation pathway is normally inactive. Flies homozygous for Hsp26-tTa with tRe–msl-2^{NOPU} (*18*) were grown on media containing tetracycline to inhibit expression of *msl-2*^{NOPU}. Small populations were transferred to normal media, and the sex ratio was scored. No female progeny were seen, in comparison to >5000 male progeny (Table 2). We also tested Yp3-tTa with tRe–msl-2^{NOPU}. As expected, combining female-specific expression of tTa with a female-specific tTa-responsive lethal also gives conditional female-specific lethality.

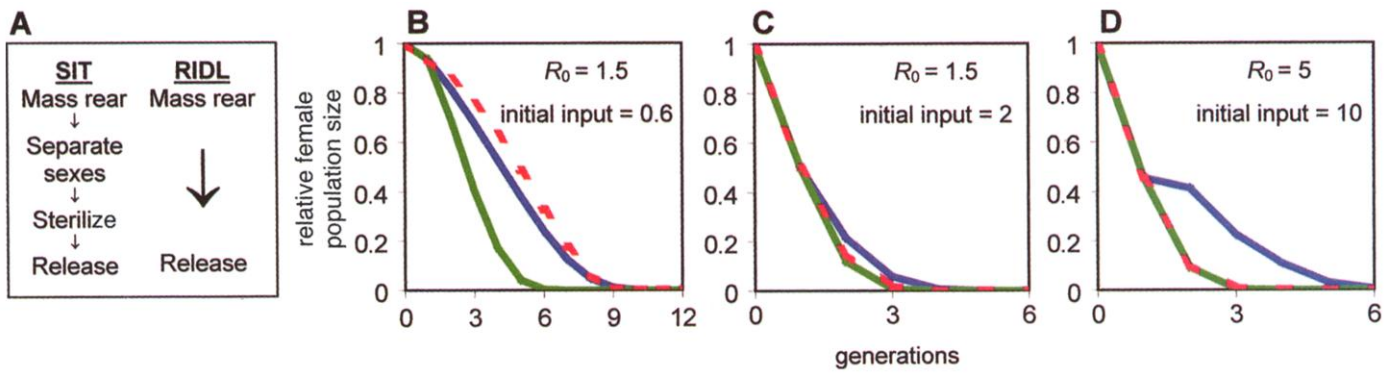

Fig. 1. Comparison of SIT and RIDL. (**A**) Outline of SIT and RIDL. In RIDL, sex separation is an automatic consequence of withdrawal of permissive conditions. No sterilization step is required. Furthermore, any life-cycle stage can be released. (**B** through **D**) Comparison of the effectiveness of SIT and RIDL. We assume that a control program releases a constant number of males per pest generation. This number is presented in relation to the initial male pest population. Each wild female mating with a fertile male produces R_o adult females in the next generation. Population size (number of females relative to initial female population size) is plotted against time (in generations) (*23*). We compare conventional SIT with three versions of RIDL. In the simplest version (blue curve), released males are homozygous for one DFL. Their female progeny die; males survive and are heterozygous for the DFL. In the second version (dashed red curve), released males additionally carry a dominant male (and female) lethal gene. This must have a sufficiently early lethal phase so that the released males themselves are not affected. As with SIT, the released males produce no viable offspring. The third version (green curve) assumes that 100% of the progeny of a DFL/+ heterozygote, rather than the normal 50%, inherit the DFL chromosome. This theoretical optimum might be approached in several ways. Released males could be homozygous for DFLs on multiple chromosomes, the DFL could be linked to a meiotic drive/segregation distortion system, or the DFL could be linked to an insecticide-resistance gene and used in combination with an insecticide program. This third version is always more effective than conventional SIT or either of the other two versions of RIDL.

We constructed a simple mathematical model to examine the relative effectiveness of SIT and several variants of RIDL (Fig. 1). Release of insects homozygous for one dominant female-specific lethal gene (DFL) was similarly effective to SIT, whereas enhanced versions of RIDL were more effective. Our model underestimates the relative effectiveness of RIDL compared to SIT because it does not account for advantages of RIDL, such as reduced production costs and the fitness advantage that transgenic males are likely to have over irradiated males. RIDL has additional advantages over SIT. Efficient removal of females from the released population, combined with marking the transgenic construct with an easily scored dominant marker, such as green fluorescent protein, would improve field trap data. The RIDL stock would produce no viable female progeny under normal environmental conditions, therefore the hazard posed by accidental release from a factory would be minimized. RIDL would also allow the factory strain to be released at any life-cycle stage, rather than requiring that the strain be grown to a late stage to allow sex separation and sterilization. We have demonstrated the system in *Drosophila*; the challenge now is to translate this to a pest of economic importance.

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17. *msl-2*^{NOPU} is a *msl-2* transcript with ablated Sxl binding sites in the 5′ and 3′ untranslated regions (UTRs) (*20*). These sites in the UTRs normally restrict the Msl-2 protein to males, where it forms a complex that hyperactivates the X chromosome as part of the dosage compensation mechanism (*21*).
18. For these experiments, recombinant second chromosomes were used, which carried the tTa and the tRe constructs, that is, Hsp26-tTa with tRe–msl-2^{NOPU} or Yp3-tTa with tRe–msl-2^{NOPU}.
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24. We thank B. Bello for the Hsp26-tTa and tRe-LacZ fly lines, M. Bownes for the Yp3 expression construct pFBE, D. Rogers for critical reading of the manuscript, and P. Burns for technical assistance. This work was funded by UK Medical Research Council (MRC) grant G117/255 to L.S.A. D.D.T. is a UK Biotechnology and Biological Sciences Research Council DPhil student, C.A.D. is supported by the Wellcome Trust, and L.S.A. is a MRC Senior Research Fellow.

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bicoid-Independent Formation of Thoracic Segments in *Drosophila*

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The maternal determinant Bicoid (Bcd) represents the paradigm of a morphogen that provides positional information for pattern formation. However, as *bicoid* seems to be a recently acquired gene in flies, the question was raised as to how embryonic patterning is achieved in organisms with more ancestral modes of development. Because the phylogenetically conserved Hunchback (Hb) protein had previously been shown to act as a morphogen in abdominal patterning, we asked which functions of Bcd could be performed by Hb. By reestablishing a proposed ancient regulatory circuitry in which maternal Hb controls zygotic *hunchback* expression, we show that Hb is able to form thoracic segments in the absence of Bcd.

In *Drosophila*, a key component of the anterior maternal system is Bcd (*1*). Embryos from mothers mutant for *bicoid* (*bcd*) lack head, thorax, and some abdominal segments. The maternal *bcd* mRNA is localized to the anterior pole of the egg and early embryo, and its translation generates an anteroposterior gradient of the Bcd homeoprotein (*2*). In the syncytial environment of the early fly embryo, the Bcd protein appears to act as a morphogen in dictating distinct developmental fates by providing a series of concentration thresholds: At low levels, Bcd acts through high-affinity binding sites to activate target genes like *hunchback* (*hb*) for the formation of the thorax, whereas high levels of Bcd activate head gap genes, such as *orthodenticle* (*otd*), which contain low-affinity Bcd binding sites (*3*). However, *bcd* seems to be the result of a recent gene duplication event in the Hox cluster of flies, which would explain why no *bcd* homologs have been identified outside higher Dipterans (*4*). Thus, different insect species may use other morphogens to pattern their embryos. One candidate morphogen that has been functionally conserved during evolution is the zinc-finger protein Hb (*5*–*7*).

In *Drosophila*, a maternal Hb gradient is established by the Nanos protein that blocks translation of the ubiquitously distributed *hb* mRNA in the posterior region of the embryo (*8*). This Hb gradient can by itself provide long-range polarity to the embryo and compensates for the absence of Bcd in the formation of abdominal segments (*6*). In addition, Hb synergizes with Bcd to pattern the anterior region of the embryo (*9*). However, the zygotic, *bcd*-dependent expression of *hb* (*10*) causes an intrinsic problem for the analysis of the specific roles of the two morphogens: Whenever Bcd activity is altered, Hb activity is changed (*3*). Thus, many of the effects attributed to Bcd might indirectly be caused by Hb. The *hb* gene is expressed from two independent promoters, P1 and P2 (Fig. 1A) (*11*): Maternal and late blastoderm expression in the central (parasegment 4, PS4) and posterior stripes are initiated at P1, whereas P2 mediates the zygotic, *bcd*-

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