judges, the immediate application to industry (or the 'responsible research and innovation' (RRI) issues, which emphasize the utility and benefit for society and the environment) should be key factors of a successful project. If they were, final rankings would certainly change and successful teams would send a clear message on the trends to follow. Judging is always, but particularly in iGEM, a bidirectional process: it ranks proposals but it also shows the way for forthcoming ones.

Given the wide range of complexity and immediate industrial applicability among iGEM projects, we suggest that the degree of sophistication (for example, the number of biological parts/devices used, the difficulty of the host organism and the complexity of regulation output) should be formally considered as a ranking criterion for judges. This would help to further increase the competitiveness of the projects.

If the competition is to place more emphasis on translating projects into real industrial applications, then more thought needs to be put into judging criteria that reinforce this aspect. At present, prizes perhaps encourage spectacular and audacious basic research, which is often not built upon; each year many teams set up brand new projects unrelated to past efforts, even award-winning ones. A greater proportion of industrial members on the judging committee would have an immediate effect by redirecting the competition from the 'game phase' (preliminary exploration) to the 'real-world phase'. More judges from government with expertise in regulatory, health, agricultural or defense issues may also expand the diversity of views and decrease academic biases. An increased presence of Asian judges in the world jamboree would also be highly desirable. Another suggestion for improving the quality of judging is standardization of the number of judges per team. Although similar numbers are assigned to each track, judges can cast votes for unassigned teams. As a result, some teams often have many more votes (either positive or negative) than others. Judging has improved a lot during the past few iGEM competitions. The online questionnaire introduced in 2012 to be filled in by judges incorporates some suggestions that arose during the 2012 regional jamborees, particularly in Europe. It is arguable whether the machine-based ranking of teams should be corrected with data such as team budget or number of students, advisors or instructors. Given the educational nature of the competition, we suggest that it should.

A greater involvement of ELSI specialists and, particularly, a focus on reflexivity and RRI would also help to shape competition trends by encouraging teams to define their projects with societal and environmental benefits as major goals, along with one of the central aspects of RRI: transparency. Transparency has always been a guiding principle in iGEM, with an open-source–like Internet-based community that shares data, protocols and DNA samples. The economic resources used in iGEM should not be excluded from such information in future. Detailed data on public and private funding as well as their precise assignment throughout the project should be a requisite for each iGEM team. As stated above, we believe that fair judgment is not possible without taking into account the funding-to-results ratio. Determining this ratio is central to assessing productivity of a particular project and of the competition as a whole. Therefore, for the sake of transparency, we propose that participating teams be asked to make their budget public on their wikis.

In summary, we have proposed a range of suggestions that could improve the quality of standards, increase transparency of funding, foster industrial orientation and redefine and enhance judging of the competition. The experience of a decade of iGEM indicates that such redefinition is imperative for this outstanding competition to meet the great expectations of synthetic biology going forward.

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Sex-ratio-biasing constructs for the control of invasive lower vertebrates

To the Editor:

There are few cost-effective means of controlling the many types of invasive fish, amphibians and reptiles that cause substantial economic and ecological damage worldwide[1]. Notable examples include sea lampreys (*Petromyzon marinus*), common carp (*Cyprinus carpio*), cane toads (*Bufo marinus*), bullfrogs (*Rana catesbeiana*) and the brown tree snake (*Boiga irregularis*)[1]. Genetic strategies based on constructs that heritably reduce female survival or fertility[2–4] could provide a solution. Here we report the first successful trials of such constructs in fish and present models suggesting that their use in combination with other strategies could lead to effective species-specific control and possible long-term eradication of such pests.

We examined two approaches to reduce effective female population sizes: female-specific sterility (FS) and female-specific lethality (FL), focusing on the FL strategy because of the successful application of this approach in insects[4]. A preliminary

assessment of an FS construct was done (**Supplementary Methods, Results** and **Supplementary Figs. 1** and **2**). The FL construct combines a female-specific promoter from vitellogenin 1 (*vtg1*) and a cell-death sequence. Vitellogenin is ubiquitous in egg-laying vertebrates, is expressed in the liver of mature females in response to female hormones and is involved in production of an egg-yolk protein precursor that is transported to maturing ova through the bloodstream[5]. We confirmed liver expression and hormone sensitivity in the model species, zebrafish (*Danio rerio*), using the *vtg1* promoter coupled to DsRed (*vtg1*-DsRed) in transient transfection assays (**Supplementary Methods**, **Supplementary Results**, and **Supplementary Figs. 3** and **4**). Zebrafish were used because of their small size and short generation time and because they are closely related to several invasive carp species, potentially facilitating transfer of the technology to these pest species.

For the FL prototype, we used the alpha chain of ricin as a lethal effector[6]. Two FL constructs were integrated into zebrafish: one contained the ricin alpha chain under the constitutive control of the *vtg1* promoter ('direct drive') (**Supplementary Fig. 5**) and the second incorporated a repressible promoter—a variant of the commercially available tetracycline/doxycycline-responsive P_{hCMV*} promoter ('Tet-off') (**Supplementary Figs. 6** and **7**). F_2 offspring in both lineages were strongly male-biased. Populations of wild-type (WT) zebrafish in our laboratory are typically 55–60% males. By comparison, 91% of the offspring of heterozygous F_1 direct-drive adults were male. Analyses of full siblings that differed in genotype confirmed the male bias for both the direct-drive and repressible constructs (**Supplementary Fig. 8**). Across all experiments, 86% of F_2 individuals with an FL construct on one chromosome (CN = 1) and 100% of those with copies on two (CN = 2) were male (**Fig. 1a**). Those with two copies included individuals homozygous at one locus and individuals heterozygous for both the direct-drive and the repressible constructs.

A strong male bias in the offspring was confirmed by using the repressible construct to rescue female carriers that could be used for breeding. At maturity, all of the carriers ($n = 9$) in the unrepressed (no doxycycline, no-dox) treatment group were male, whereas 8 out of 17 carriers (44%) in the dox treatment group were female. All the females had large gonads full of late-stage ova. The proportion of females among carriers in the dox treatment differs significantly from that in the no-dox treatment (Fisher's exact test, $P < 0.01$) and is within the range we observed for WT fish (40–45%), confirming successful repression of the lethal sequence. We used these rescued females and their progeny to breed a small number of repressible FL carriers through to F_4; reared without dox, all 13 F_4 individuals were male.

We tested the direct-drive FL construct in transient assays in the invasive pest *C. carpio* (common carp) and obtained results consistent with enhanced female mortality (as seen in *D. rerio*). Of 27 mature carp from eggs that had been electroporated

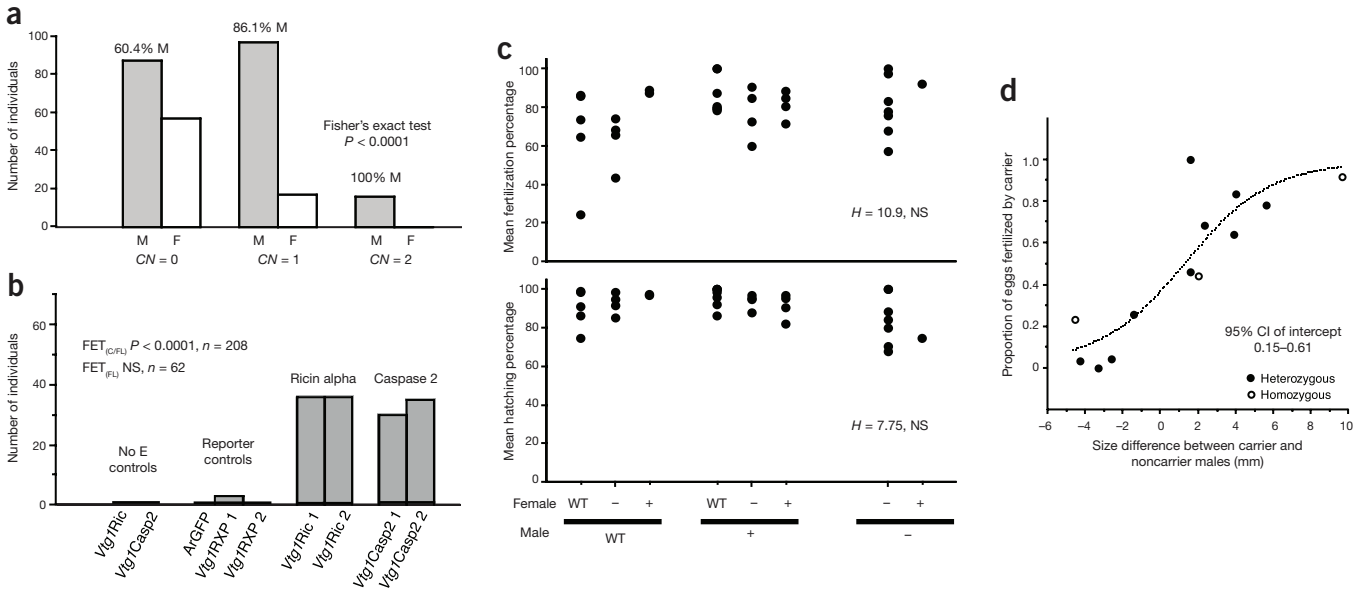

Figure 1 Effects of the FL constructs on sex ratios and reproductive parameters in zebrafish and common carp. (**a**) Summary of data from all direct drive–and repressible female-specific lethality–construct experiments of sex ratios of zebrafish that differ in the number of chromosomes (CN) with integrated constructs. Total $N = 273$. (**b**) Mortality rates of 7-month-old juvenile common carp that had been transiently transfected (that is, ova and sperm were transfected prefertilization) with FL constructs containing ricin alpha or caspase 2 as the cell death sequence or reporter genes (reporter controls). Constructs were triggered in the treatment groups by dosing rearing water with estradiol and compared to similarly electroporated fish in the absence of estradiol (no E controls). (See **Supplementary Methods** for details.) The numbers '1' and '2' denote two groups of fish in each experiment that were independently treated in separate aquaria. Total $N = 208$. Differences between the pooled controls and pooled FL variants are significant at $P < 0.0001$ (Fisher's exact test, $FET_{(C/FL)}$); differences between the two FL variants ($FET_{(FL)}$) are not significant (NS). (**c**) Pair-mean fertilization and hatching success rates for each of three different male genotypes (wild type = WT; construct-positive (+) and construct-negative (–) siblings) bred with females of three different genotypes (as above). Data were collected for 30 pairs of randomly assorted fish and include results from 192 breeding events. Significance tested using Kruskal-Wallis H statistic. (**d**) Carrier male mating success (the proportion of eggs fertilized) in 13 pair-wise competition trials as a function of the difference in size (standard length) between carrier and WT males. The proportion of fertilizations attributable to the carrier was a strong nonlinear function of the size difference between it and the paired noncarrier ($R^2 = 0.56$, $P = 0.003$, proportional fertilization success logit transformed before regression; dotted line indicates fitted function); mating success that correlates with male size is common in fish. The predicted success rate for carriers when the competing males were a similar size was not significantly different from 50% (95% CI of the intercept of the size difference/fertilization success regression 15–61%). Preliminary data for three CN 2 male carriers (open symbols) suggest no reduction in competitiveness at higher copy numbers.

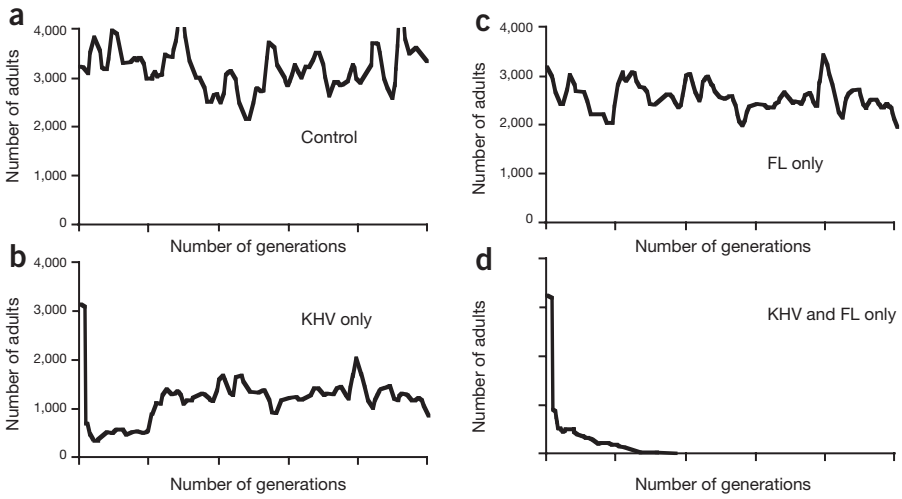

Figure 2 Potential synergy between the release of a sex-biasing gene construct and classic biological control. (**a**–**d**) Modeled dynamics of a realistically parameterized carp population (**a**) as compared to the same population subject to the release of KHV alone (**b**), stocking of FL carriers alone (**c**) and a combined release of both (**d**). Model details are provided by ref. 3 and summarized in the **Supplementary Methods**. We assume a generation time of 4 years, a maximum age (99% mortality) of 20 years, moderate levels of density dependence and environmental variability and no effect of released fish on development of the resistance to the virus. Both control strategies are started 3 years into the simulation. The KHV simulation assumes 80% mortality in year 1, 50% in year 2, 30% in year 3 and 20% per year thereafter, uniformly distributed across age classes and sex. These values are based on observed mortality rates in the field and attenuation as less resistant fish are eliminated from the population[11]. Stocked FL carriers are assumed to be resistant to KHV, and thus accrue an annual 20% mortality due to the virus, in addition to the species-normal age-dependent mortality schedule (see details in ref. 3). The FL simulation assumes one copy of the construct is lethal to females, an annual stocking of juvenile male carriers equivalent to 3% of the pre-intervention recruitment and four independently segregating copies of the construct.

with the construct, 21 (78%) were male (**Supplementary Fig. 9**). Only half (18 out of 36) of their siblings that had been electroporated with a reporter gene (*vtg1*-RXP) control construct were male. The difference in sex ratios between the treatment and control groups is significant at $P < 0.01$ (χ^2 contingency = 8.33, d.f. = 1). Using ricin alpha chain as a lethal effector could raise regulatory and public acceptability issues, so we used transient assays in carp to test the coding region of the fish apoptosis gene *Caspase2* (*Casp2*)[7] as a possible alternative (**Supplementary Fig. 10**). We electroporated carp eggs with *vtg1*-Ric, *vtg1-Casp2* and, as a control, *vtg1*-RXP and compared mortality rates of 7-month-old juveniles after they had been dosed with estradiol in aquarium water, which stimulates *vtg1* expression in fish before their normal sexual maturation[8]. Mortality rates were similar for the groups treated with ricin alpha and Casp2 FL (36% and 33%, respectively; Fisher's exact test, not significant) and were significantly increased for both compared with any of three control groups ($P < 0.0001$ for pooled controls and pooled estradiol-dosed FL carriers) (**Fig. 1b**).

The utility of the FL construct for pest management depends on the fitness of male carriers. At maturity, the ratio of CN 0, CN 1 and CN 2 male offspring of a pair of heterozygous F_1 direct-drive zebrafish was not significantly different from that predicted by Mendelian inheritance ($\chi^2 = 0.67$, d.f. = 2, $P > 0.5$), implying little or no extra mortality due to the construct in laboratory conditions (**Supplementary Fig. 11**). Fertilization and hatching rates did not differ between WT and construct-negative or construct-positive, full sibling, direct-drive F_2 males, irrespective of whether they were bred with WT or carrier females (**Fig. 1c**). The mating competitiveness of male carriers and noncarriers did not differ significantly when they competed for fertilization of eggs produced by a WT female, and it was similar for CN 1 and CN 2 carriers (**Fig. 1d**). Growth rates for full-sibling carriers and noncarriers were not significantly different (Kruskal-Wallis $H = 0.66$, not significant) (**Supplementary Fig. 12**). Carriers and noncarriers also seemed to have similar levels of activity, had similar life spans and did not differ noticeably in terms of sensitivity to stress or pathogens (data not shown).

Modeling and cage trials of short-lived insects indicate that control programs based on release of FL carriers alone can reduce pest numbers to the point of possible eradication[2–4]. However, because of their longer life spans, eradication of vertebrates may take longer and be logistically more demanding, which could discourage use of the techniques[9]. The models also suggest that the efficacy of constructs that bias sex ratios can be enhanced by complementary management actions that reduce the number of WT individuals against which stocked carriers compete or that remove long-lived WT individuals from the population (e.g., **Supplementary Fig. 13**).

We explored this synergy by simulating and comparing the effects of control programs targeting common carp based on releasing fish carrying FL constructs, on koi herpes virus (KHV, a nominally *C. carpio*–specific virus being considered as a classic biological control agent for use against common carp in Australia[10]) and on both together. The best available estimates suggest initial mortality rates due to KHV of 70–80%, followed by attenuation over time as the proportion of resistant fish in the population increases[11]. As a consequence, modeled carp populations fall by 70–80% in the decade following release of KHV, but gradually return to 30–40% of pre-release levels (**Fig. 2a**,**b**), the exact value depending on the long-term susceptibility of the pest population to the virus. Annual stocking of FL carriers at a moderate and logistically feasible level (3% of average recruitment before intervention) and with a low number of independently segregating copies (in this case, 4) is predicted by modeling to have a negligible effect on the pest population (**Fig. 2c**). For this analysis, we deliberately chose a stocking rate and copy number that had minimal impact of the target population, based on our previous modeling[3]. However, modeling of the simultaneous use of the FL construct and KHV predicts eradication of carp in fewer than ten generations (**Fig. 2d**); the combined effect of the two approaches is much greater than the sum of their individual impacts. Population levels depressed by the virus greatly enhance the introgression of the FL construct into the remaining population, rapidly skewing sex ratios and sharply reducing female numbers, population fertility and recruitment.

Preliminary studies on biological control agents and gene constructs that reduce female numbers or fertility have been carried out on cane toads in Australia[12] and are proposed for invasive bighead carps (*Hypophthalmichthys* spp.) in North America[13]. Transferring the FL or FS technology to either pest would be fairly

simple, given that the genes involved are ubiquitous among egg-laying vertebrates, and there are well-developed transfection methods for amphibians[14] and fish. Integrated management involving FL, FS and other sex-ratio-distorting genetic options[15] coupled with classic biological control could prove useful against various lower vertebrate pests. Even so, for many vertebrate pests, finding a suitable biological control agent has proved difficult. Such agents also often result in substantial economic and environmental damage[16]. Sex-ratio-distorting genetic technologies have the potential for high specificity and could be combined with strategies that are more precisely and reliably targeted than classic biological control agents to achieve effective pest management. Such strategies might include novel species-specific biocides, disrupting spawning activity, physical removal of pests or releasing carriers during or immediately after periods of natural low breeding success or high mortality, for example, during droughts[9]. The combination of population depression using conventional approaches and the long-term decrease in pest reproductive potential using genetic options provides an opportunity to manage effectively and safely vertebrate pests that are currently considered intractable.

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