

Reply from L.G. Harshman and A.A. Hoffmann

It has been argued that laboratory evolution experiments are superior to comparative studies^{1,2} and phenotypic manipulations³ for the study of evolution. It has also been argued that there are constraints on the design of most field evolution studies compared with laboratory evolution experiments³, and suggested that interpreting the results of field evolutionary studies is problematical relative to laboratory evolution experiments³. However, a crucial perspective on laboratory evolution studies calls these points of view into question. As we discussed in our recent *TREE* article⁴, there is a range of problems associated with laboratory selection experiments using *Drosophila* to study the evolution of life history and stress-related traits. In their letter, Matos *et al.*⁵ did not significantly address any of the issues we raised⁴.

Their reply⁵ is a bit puzzling because their argument is similar to the one we presented⁴. Like us, they describe the problem associated with conducting selection experiments using populations recently established in the laboratory. Specifically, we stated that 'There is some evidence that *Drosophila* can adapt

rapidly to laboratory culture⁶, reducing the concern about using populations maintained in the laboratory for only a short term. Nevertheless, it is unclear how many generations are required to dampen any spurious correlations potentially generated by domestication. To illuminate this issue, it is important to investigate the process of *Drosophila* domestication in terms of the time course of changes in life history and of stress-related traits, and to investigate the patterns of genetic correlations among traits⁷. Once we have such information, it can be used as a basis to decide how long to maintain populations in the laboratory before initiating selection experiments; recommendations can be made about any likely confounding effects; and general guidelines could be established for comparing recently established and long-established populations. In this context and with a retrospective emphasis on 'relatively', we suggested that 'Ideally, it might be desirable to conduct separate selection experiments on a long-standing equilibrium population and on a population derived relatively recently from the field.'

Lawrence G. Harshman

School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588-0118, USA (lharsh@unlserve.unl.edu)

Ary A. Hoffmann

Dept of Genetics and Evolution, La Trobe University, Bundoora, Victoria 3983, Australia (genaah@gen.latrobe.edu.au)

References

- 1 Lauder, G.V. *et al.* (1993) Adaptations and history. *Trends Ecol. Evol.* 8, 294–297
- 2 Leroi, A.M. *et al.* (1994) What does the comparative method reveal about adaptation? *Am. Nat.* 143, 381–402
- 3 Rose, M.R. *et al.* (1996) Laboratory evolution: the experimental wonderland and the Cheshire cat syndrome. In *Adaptation* (Rose, M.R. and Lauder, G.V., eds), pp. 221–241, Academic Press
- 4 Harshman, L.G. and Hoffmann, A.A. (2000) Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* 15, 32–36
- 5 Matos, M. *et al.* (2000) An evolutionary no man's land. *Trends Ecol. Evol.* 15, 206
- 6 Frankham, R. and Loebel, D.A. (1992) Modeling problems in conservation genetics using captive *Drosophila* populations: rapid genetic adaptation to captivity. *Zool. Biol.* 11, 333–342
- 7 Matos, M. *et al.* (2000) Adaptation to the laboratory environment in *Drosophila subobscura*. *J. Evol. Biol.* 13, 9–19

PERSPECTIVES

Transgenic organisms in evolutionary ecology

Marc Tatar

Evolutionary ecology aims to understand how phenotypes are designed for reproductive success and survival. Perhaps the most powerful approach towards this goal is to alter a character genetically and observe the resulting change in reproduction, survival, growth, defense or competitive ability. Until recently, this strategy was not practical. Transgenic manipulation now offers a solution – novel genes are introduced into the germ line and are then expressed in the developing organism. This technique is already available in model and agricultural organisms. The challenge for molecular evolutionary ecologists is to find ways to adopt these powerful systems to understand the mechanisms underlying adaptive traits and their evolution.

Marc Tatar is at the Dept of Ecology, Evolution and Behaviour, Box G-W, Brown University, Providence, RI 02912, USA (marc_tatar@brown.edu).

phenotypes of most physiological and cellular processes are specifically molecular, and it is difficult to relate variation at this level to fitness: how do we test whether the tertiary protein shape of the α -hemoglobin is an adaptation? Furthermore, to analyse adaptation we must measure the fitness of alternative phenotypes across a spectrum of relevant environments. But it might not be possible to manipulate natural variation to fit this design. Finally, many traits are likely to be constrained by pleiotropy and adaptation is a compromise when viewed from the perspective of a single trait. Yet, our understanding of pleiotropy has been limited by the quality of standing variance and covariance. For instance, life histories evolving under experimental selection often are accompanied by negatively correlated selection responses, but this observation cannot specify which traits functionally constrain fitness³.

One solution is to produce novel variation experimentally, either through phenotypic or genetic manipulation⁴. In particular, transgenic technology provides the ability to introduce genes, to alter gene copy number, to control ectopic gene expression and even to generate directed mutations at specific loci. In the research presented here (reviewed and extended

The tools available to understand how natural selection shapes adaptation are diverse^{1,2}: artificial selection, comparative methods, phenotypic selection analysis, and the analysis of experimental association between selection gradients and environments. Working with pheno-

typic and genetic variation among individuals and groups, these approaches collectively demonstrate how and why some traits are adaptations to different environments. But for some phenotypes, such as elements of morphology, variation is limited or nonexistent. In addition, the

Table 1. Methods to introduce transgenes in plants^a, invertebrates^b and vertebrates^c

Method	Procedure	Taxa
Physical placement		
Microinjection	Direct injection of DNA into the pronucleos or perinuclear cytoplasm of the fertilized egg.	Insects, fish and mammals
Electroporation	Electric pulse field drives DNA into the embryo or embryonic stem cells, or into plant cell protoplasts established from cell lines.	Fish, rodents and plants
Biolistics	Bombardment of eggs, embryo or tissue culture with DNA-coated particles.	Plants
Lipofection	Vector packaged in lipid micelles, which fuse to the egg or to plant cell protoplasts.	Rodents and plants
Sperm-mediated transformation	Sperm coated with DNA vectors fertilize the embryo.	Rodents, sea urchin and chicken
Infective vectors		
Retrovirus	Expose embryos to recombinant retrovirus.	Birds
<i>Agrobacterium</i>	Tumor-forming bacterium with recombinant, tumor-inducing plasmid is cultured with totipotent root tissue. Generates stable transformation with direct integration into host genome.	Plants
Symbionts	Fungal endophytes. Gut bacterial endosymbionts.	Turfgrass <i>Tsetse</i> and <i>Rhodnius</i>

^aData taken from Refs 26–28.

^bData taken from Refs 29–32.

^cData taken from Refs 29,33.

Box 1. Terms of transgenic technology

Background effects: the phenotypic effect of transgene expression depends upon interactions of the gene product with the products of other host genes. Genetic variation among individuals produces variation in the background of epistatic interactions with the transgene.

Extopic expression: protein expression derived from transgenic cDNA. The cDNA might be for genes already present in the organism or for novel genes.

Insert mutagenesis: mutations in host DNA are caused at the integration of the transgene. They can disrupt host transcription.

Integration sequence: DNA sequences of the recombinant vector construct that are responsible for excision of the cDNA from the vector, with incorporation of the cDNA into the host chromosome. Often these are inverted repeats derived from transposons.

Position effects: the level of transgene expression varies with the site of integration. Caused by variation in promoter activity dependent upon neighboring DNA sequences.

Recombinant vector construct: an engineered fragment or plasmid of DNA used to replicate, transport and integrate transgenes.

Selection marker: a transgene engineered to coincide with the cDNA of interest, which is used to determine when an organism has incorporated the cDNA. Often these genes code for pigments, or antibiotic or herbicide resistance.

Site-specific recombination: crossing-over induced to occur at specified DNA sequences. With experimental specificity it is used to insert or excise transgenes, to induce chromosome exchange and to direct mutations at desired loci. This procedure is catalysed by recombinase proteins and involves transgenic recombinase target sequences derived from yeast or prokaryotes, or of DNA homologous to the host target.

Stable and/or unstable transfection: extrachromosomal transgenes introduced into cells are unstable because they can fail to be represented in all daughter cells or progeny. Transgenes that integrate into the host chromosome are stable and heritable.

Transgene co-placement: alternative transgenes are inserted as alleles into the same locus of integration.

Transgene: new DNA acquired by eukaryotic organisms. The source might be from the same species or from different taxa.

from the 1997 ASN symposium on ‘Experimental Approaches to Testing Adaptation’⁴), we see how transgenes can be used to manipulate phenotypes across relevant environments, to produce variation at the level of protein phenotypes and to uncover the molecular basis of trade-offs. Each case reveals adaptive features of traits that could not be discovered from standard methods.

Transgenes

Transgenes are transcribed nucleotide sequences originating from the same or different species, which are introduced artificially into the genome of an organism. Transgenes are often carried by a recombinant vector construct (Table 1), which can include integration sequences, a promoter, a selectable marker and, in some cases, regulatory or homologous sequences to manipulate expression of the transgene after its integration (see Box 1 for terms of transgenic technology). The aim of transgenic analysis is to alter a specific phenotype from the bottom up; transgene transcription must affect the developmental, metabolic, regulatory or housekeeping pathways thought to underlie the traits that we test for associations with fitness. This goal can now be accomplished by ever more elegant techniques that control for position, background and mutational effects, while regulating temporal expression (Table 2). The genetic aim is to reliably increase or decrease the amount of target gene product without inadvertently disrupting other phenotypes. Although specific techniques are needed to make transgenic organisms, many are available in model organisms designed for molecular studies of development, genetics, medicine and agriculture. With these materials, the challenge for evolutionary biologists is to recognize which genes offer an avenue to study fitness. Indeed, in the following cases, except for *Arabidopsis thaliana* herbicide resistance, researchers made use of existing transgenic organisms.

Heat-shock response

Many organisms experience dangerous thermal conditions. Stress induction of heat-shock proteins (Hsp) has long been thought to be one functional adaptive feature of organisms to prevent ensuing damage from altered or denatured proteins⁵, because Hsp can be undetectable until organisms are stressed, after which organisms present a level of acquired tolerance that correlates with levels of Hsp. However, these correlations cannot show that the heat-shock response is a proximate cause of thermotolerance or that the response is adaptive with respect to ecologically relevant challenges.

Table 2. Recombinant vectors used to integrate, regulate and manipulate transgenes in plants and invertebrates

System	Mode of integration and control	Construct property	Refs
<i>P</i> -element	<i>Drosophila</i> transposon is engineered into a plasmid vector. Transgenes placed between the <i>P</i> -element terminal repeats are integrated into the host genome by action of transposase.	High single copy integration rate. In simplest form, it lacks control of expression or integration site.	31
<i>Ti</i> plasmid	The tumor-inducing (<i>Ti</i>) plasmid of <i>Agrobacterium tumefaciens</i> transfers part of the <i>Ti</i> genome (T-DNA) into the genome of an infected plant. T-DNA can be engineered to replace oncogenes with cDNA for ectopic expression.	In many plants, high single copy integration rate. In simplest form, it lacks control of expression or integration site.	34
FLP- <i>FRT</i>	Site-specific recombination following stable transformation. Elements are derived from yeast but function in many eukaryotes. Yeast FLP recombinase (FLP) catalyses recombination between FLP recombination targets (<i>FRT</i>). Transgenes are engineered with flanking <i>FRT</i> -sites, which produce site-specific duplications and deletions when passed through an FLP-producing strain.	Site-specific deletion and duplication of transgene. Provides control for insert mutagenesis.	8,35
FLP-out	Site-specific recombination following stable transformation. In the transgenic construct a pair of <i>FRT</i> -sites that sandwich a stop-transcription sequence separates a constitutive promoter from the cDNA. Transgenic FLP under inducible control is introduced elsewhere in the genome and activated experimentally, the <i>FRT</i> -flanked sequence 'flips-out' to permit transcription of the cDNA.	Manipulated excision of negative control region. Provides controls for insert mutagenesis and regulates when ectopic expression begins.	36
Cre- <i>loxP</i> with FLP- <i>FRT</i>	Site-specific recombination following stable transformation. In eukaryotes, bacteriophage P1 Cre recombinase (creates recombination) excises DNA intervening between target <i>loxP</i> sites ['locus of crossingover(x), P1']. In <i>Drosophila</i> , the <i>P</i> -element transformation vector contains one transgene sandwiched between <i>loxP</i> sites and the second transgene sandwiched between <i>FRT</i> -sites. Initially, alternate transgenes are placed at the same site. Subsequently, one is eliminated selectively by genetic passage through either Cre-producing or FLP-producing strain.	Coplacement of alternative transgenes with subsequent controlled excision of one gene. Cre- <i>loxP</i> alone can also be used for site-specific integration.	25
UAS-GAL4	The transformation vector contains a yeast upstream activating sequence (UAS) engineered with target cDNA. In <i>Drosophila</i> , many independent lines are available that transgenically express yeast activator protein GAL4 in tissue-specific patterns. In those cells, GAL4 induces expression of UAS-cDNA constructs.	Organism is a mosaic with the transgene expressed only in a specified set of tissues. Can be combined with FLP-out system.	37
Integrated extra-chromosomal arrays	With hermaphroditic <i>Caenorhabditis elegans</i> , transgenes are microinjected directly into gonads. These are unstable and can be lost through subsequent generations and cell lineages. Transgenes can be integrated into the worm genome by irradiation of the unstable strain and progeny are screened over two generations for stable inheritance.	Integrates transgenes into worm genome. Low efficiency of integration.	38

Inducible stress tolerance can arise from other mechanisms that are coexpressed with Hsps, including osmotic protectants, modification of cell membranes and the expression of enzymes⁵.

Feder *et al.* definitively established the impact of Hsps upon organismal stress tolerance with transgenic *Drosophila melanogaster*⁶. The most abundant Hsp in the fly is stress inducible Hsp70, which has been studied widely as a model of eukaryotic gene regulation⁷. Feder *et al.* used matched transgenic strains transformed and subsequently manipulated by homologous recombination to include 12 copies of inducible *Hsp70* genes in addition to the fly's native ten copies⁸ (FLP-*FRT* system; Table 2). In the 'extra-copy' strain, heat induces overexpression of Hsp70 protein relative to the level produced by the 'excision' control strain. Tolerance to severe heat stress was enhanced in transgenic flies expressing the additional Hsp70. Furthermore, Hsp70 overexpression was induced in larvae at

ecologically relevant temperatures (i.e. those experienced in rotting orchard fruit⁹). The survival benefits of Hsp70 at temperatures where selection can act upon variation for this protein argue that inducible Hsp70 protein underlies an adaptation to thermal stress¹⁰. But why is this gene inducible rather than constitutively expressed? The studies of transgenes offer opportunities to discover relevant trade-offs. In the case of Hsp70, transgenic extra-copy larvae had reduced growth and survival rates in the absence of heat stress¹¹. Regulated induction might evolve because Hsp70 expression defends against potentially lethal challenges to larvae but interferes with development under ambient environmental conditions.

Life history and stress resistance

Resistance to acute stress is a specific component of fitness affected by the heat-shock response. By contrast, age-specific survival is a general characteristic that contributes to demographic com-

ponents of fitness. Mechanisms to maintain somatic function are underlying features of life history, which are optimized within the constraints of reproductive demands¹². The outcome of this trade-off between survival and reproduction is senescence. Now, biogerontologists are using transgenes to evaluate this model, and these data might provide fundamental insights on the design and genetics of life histories.

Two studies with different transgenic designs found that overexpression of superoxide dismutase (SOD) increases longevity in *D. melanogaster*. SOD alters superoxide ions to hydrogen peroxide, which, in turn, is reduced to water by catalase. Cumulative, age-dependent damage produced by unreacted superoxide is postulated to limit organismal survival and function. Parkes *et al.*¹³ used the UAS-GAL4 system (upstream activation site UAS and its yeast transcriptional activator GAL4; see Table 2) to show that constitutive overexpression of a single

human superoxide-1 (SOD1) gene in fly motorneurons was sufficient to increase adult lifespan by up to 40% relative to nonexpressing controls. Sun and Tower¹⁴ produced similar results with a binary transgenic system (FLP-out; Table 2), where transgene overexpression was limited to adults. Overexpressed fly copper/zinc-superoxide dismutase (Cu/Zn-SOD) throughout adult tissue increased stress resistance and longevity by up to 48%. In addition to manipulating the time of transgene transcription, a benefit of FLP-out is strong control for positional and background effects. Thus, with the insertion of the construct into multiple genetic backgrounds, Sun *et al.* showed that epistatic interactions are crucial to the effect of Cu/Zn-SOD on longevity. The potential fitness costs to overexpression of Cu/Zn-SOD remain to be determined.

Hsp70 and elongation factor-1 α also have been evaluated with overexpression of transgenes to determine whether these proteins contribute to survival. The use of transgenically amplified elongation factor-1 α was a pioneering effort that failed to find an effect of the gene upon senescence, but gave important insight into how the application of this technique relates to life histories¹⁵. As we might expect from complex, quantitative traits, reproduction and longevity are sensitive to where the transgene is inserted. Insertion at certain sites can produce disruptive mutations in active genes, which negate the beneficial effects of the transgene. The integration site can also determine the level of transgene transcription, and such positional effects might be larger than allelic differences of the transgenes themselves. For example, the position of integration was the major determining factor of the alcohol dehydrogenase (Adh) activity of transgenes from *D. melanogaster* and *D. affinis* *juncta*, which were co-placed with a novel Cre-*loxP* system (phage recombinase Cre affects locus of crossing over *loxP*; see Table 2)¹⁶.

Thus, an important feature of the Hsp70 extra-copy-excision system is the ability to measure rates of senescence within a genotype under heat conditions where Hsp70 will and will not be overexpressed. An effect of the Hsp70 transgene upon senescence is revealed when the improvement in mortality upon Hsp70 expression is greater for flies with extra copies of *Hsp70* compared with the flies expressing the wild-type complement of ten copies^{17,18}. In accordance with this test, subsequent to a brief heat shock, extra-copy flies aging at normal temperatures reduced their death rates by as much as twofold, although those of excision flies were unaffected. Hsp70

protein increases a component of demographic fitness – age-specific survival. Furthermore, the manipulation of Hsp70 offers the opportunity to directly test the antagonistic pleiotropy model for the evolution of senescence. Following brief heat shock the number of eggs produced by females is not reduced relative to unheated females¹⁸, but heat shock reduces the viability of eggs. The Hsp70 extra-copy-excision system of *D. melanogaster* might be used to test whether expression of Hsp70 is responsible for this negative maternal effect.

Alternatives to overexpression

One drawback of transgene overexpression as a tool for the study of adaptation can occur when the gene of interest is embedded in a linear metabolic pathway. The biochemical control model of Kacser and Burns suggests that levels of expression produced by wild-type alleles are already at a point where further activity yields greatly diminished returns^{18,19}. Thus, some classes of genes produce only a modest change in phenotype flux, in spite of potentially large increases in activity by transgenic overexpression. Other strategies of transgene analysis are not limited in this way. Rather, transgenes can be used to validate the phenotypes attributed to mutant alleles, or transgenes might be used to qualitatively change development or signaling pathways.

Arabidopsis thaliana isolated with a point mutant (*Crs1-I*) for acetolactate synthase (ALS) are resistant to the herbicide chlorsulfuron, but these plants are less fecund than plants with wild-type ALS alleles²⁰. The antagonism of these phenotypes with respect to fitness might help explain why resistance is relatively uncommon in populations rarely exposed to herbicides. However, plants isolated with the *Crs1-I* mutant might have reduced fecundity for other reasons, such as if genes for seed set or flowering are themselves polymorphic and tightly linked to the ALS locus. A powerful test to verify pleiotropy of mutant alleles is to transgenically insert a wild-type allele into a mutant organism or a dominant mutant allele into a wild-type organism. Phenotypes that are genetically complemented by the transgenic allele can be ascribed unambiguously to the function of this gene. Bergelson *et al.* used transgenic complementation to confirm the pleiotropic effects of *Crs1-I* (Ref. 20). Transgenic alleles of *Crs1-I* were introduced into plants with wild-type alleles. These plants gained chlorsulfuron resistance, but when grown in field settings they had reduced seed set relative to controls. This deleterious effect of *Crs1-I* opens the way to understand the mechanism

underlying costs of herbicide resistance. Plants transgenic for *Crs1-I* were found to have high activity levels in the catalytic step that synthesizes three branched-chain amino acids. However, seeds of these plants contained elevated concentrations of all amino acids, even from pathways where ALS is not known to play a role²¹. It is thought that ALS overactivity is responsible for detoxification of chlorsulfuron herbicide, but through unknown mechanisms it also produces a metabolic load in flowers, which results in reduced seed set.

Transgenes can manipulate large-scale traits, such as morphology, life cycles (e.g. diapause) and phenotypic plasticity, because many phenotypes are regulated at junctures in developmental or molecular signaling. At these steps, transgenes can be used to interfere, thus producing alternative phenotypes expressed under novel conditions. Schmitt *et al.*²² used this approach with tobacco to demonstrate how the phenotypically plastic shade response affects reproductive success. Many plants display stem elongation in response to crowding or shading. This plasticity is mediated by several phytochromes, but is blocked when phytochrome A (PHYA) is at high levels. PHYA is abundant in etiolated seedlings, where it regulates early growth, until it is degraded and downregulated by light. To determine whether phytochrome-mediated shade avoidance is adaptive, it is necessary to demonstrate reduced fitness in plants lacking the response under conditions where it is normally expressed. Schmitt *et al.* used transgenic tobacco ectopically expressing oat PHYA that were unable to elongate when shaded. As predicted, plants with blocked shade response displayed reduced relative fitness, measured as dry biomass, when grown in dense stands of shade-responding wild-type plants, compared with those grown in pure stands of manipulated plants. Other trials with mutant *Brassica*, which constitutively express stem elongation, were used to demonstrate the cost of elongation in the absence of crowding. Schmitt *et al.* conclude that plasticity itself is an adaptive trait, because it increases fitness by producing the appropriate phenotype across varying environments.

Molecular horizons: promises and limits

The application of transgenes to study adaptation is powerful but not without limitations. Techniques are best developed for laboratory and domestic organisms that have evolved recently under artificial conditions. How then do we specify the relevant environments in which to

assess fitness? The problem for life history traits is especially acute because age-specific survival and fecundity are sensitive to temperature, nutrients and crowding, but we rarely understand the natural ecology of these features for transgenic animal models. The work of Feder *et al.* is unique in showing not just that Hsp70 increases whole animal thermotolerance, but also in finding the larval environments in the field where Hsp70 expression affects survival. With weedy plants, such as *A. thaliana*, the ecological issue can also be resolved by planting in experimental fields and in natural sites. Technical challenges are a further limitation for this program. Control is required for alterations of genetic background, position effects upon transgene transcription and insert mutagenesis. Even in model organisms the effort to achieve this goal is substantial and involves the use of both advanced constructs and replicated transgenic introductions.

This review illustrates the potential benefits of coping with these challenges. In standard analyses of adaptation we associate natural phenotypic variation with differences in survival and reproductive success. This describes how selection can affect traits within their current range of phenotypes. Transgenic manipulation permits us to measure the fitness of traits when they are expressed outside the wild-type range. When the transgenic phenotype has low fitness in the relevant environments, we can conclude that the wild-type value is an adaptation. Transgenes also pinpoint the contributions specific genes or alleles make to fitness, and this specificity permits us to identify the pleiotropic traits that might constrain evolution. Once the genetic basis of an adaptive trait is identified we can measure how variation in promoter and coding regions or in gene copy number is distributed among populations and individuals. The fitness of specific variants can then be studied through methods of population genetics, including analysis of genotype-phenotype associations²², of gene frequency change along ecological gradients or under selection^{23,24} and of experimental competition between transgenically co-placed alleles²⁵. These approaches would then draw near to a central goal in evolutionary biology – to show how natural selection shapes adaptation by the potential change in the frequency of genes with differential effects on the components of reproductive success.

Acknowledgements

Colin Purrington and Johanna Schmitt provided thoughtful discussion. M.T. was supported by funds from the National Institute on Aging (R01 AG16632) and the American Federation of Aging Research.

References

- Rose, M.R. and Lauder, G.V., eds (1996) *Adaptation*, Academic Press
- Wade, M.J. and Kalisz, S. (1990) The causes of natural selection. *Evolution* 44, 1947–1955
- Clark, A.G. (1987) Senescence and the genetic-correlation hang-up. *Am. Nat.* 129, 932–940
- Schmitt, J. (1999) Introduction: experimental approaches to testing adaptation. *Am. Nat.* 154 (Suppl.), S1–S3
- Feder, M.E. and Hofmann, G.E. (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu. Rev. Physiol.* 61, 243–282
- Feder, M.E. *et al.* (1997) Effect of engineering Hsp70 copy number on Hsp70 expression and tolerance of ecologically relevant heat shock in larvae and pupae of *Drosophila melanogaster*. *J. Exp. Biol.* 119, 1637–1844
- Parsell, D.A. and Lindquist, S. (1994) Heat shock proteins and stress tolerance. In *The Biology of Heat Shock Proteins and Molecular Chaperones* (Morimoto, R.I. *et al.*, eds), pp. 457–493, Cold Spring Harbor Press
- Welte, M.A. *et al.* (1993) A new method for manipulating transgenes: engineering heat tolerance in a complex, multicellular organism. *Curr. Biol.* 13, 842–853
- Krebs, R.A. and Feder, M.E. (1997) Natural variation in the expression of the heat-shock protein Hsp70 in a population of *Drosophila melanogaster*, and its correlation with tolerance of ecologically relevant thermal stress. *Evolution* 51, 173–179
- Feder, M.E. (1999) Engineering candidate genes in studies of adaptation: the heat-shock protein Hsp70 in *Drosophila melanogaster*. *Am. Nat.* 154 (Suppl.), S55–S66
- Krebs, R.A. and Feder, M.E. (1997) Deleterious consequences of Hsp70 overexpression in *Drosophila melanogaster* larvae. *Cell Stress Chaperones* 2, 60–71
- Kirkwood, T.B.L. and Holliday, R. (1979) The evolution of ageing and longevity. *Proc. R. Soc. London Ser. B* 205, 531–546
- Parkes, T.L. *et al.* (1998) Extension of *Drosophila* lifespan by overexpression of human SOD1 in motorneurons. *Nat. Genet.* 19, 701–704
- Sun, J. and Tower, J. (1999) FLP/FRT-mediated induction of Cu/ZnSOD transgene expression can extend the life span of adult *Drosophila*. *Mol. Cell. Biol.* 19, 216–228
- Kaiser, M. *et al.* (1997) P-element inserts in transgenic flies: a cautionary tale. *Heredity* 78, 1–11
- Siegal, M. and Hartl, D. (1998) An experimental test for lineage-specific position effects on alcohol dehydrogenase (Adh) genes in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 15513–15518
- Tatar, M. *et al.* (1997) Chaperoning extended life. *Nature* 390, 30
- Tatar, M. (1999) Transgenes in the analysis of lifespan and fitness. *Am. Nat.* 154 (Suppl.), S67–S81
- Kacser, H. and Burns, J.A. (1981) The molecular basis of dominance. *Genetics* 97, 639–666
- Bergelson, J. *et al.* (1996) Costs of resistance: a test using transgenic *Arabidopsis thaliana*. *Proc. R. Soc. London Ser. B* 263, 1659–1663
- Purrington, C.B. and Bergelson, J. (1999) Exploring the physiological basis of costs of herbicide resistance in *Arabidopsis thaliana*. *Am. Nat.* 154 (Suppl.), S82–S91
- Schmitt, J. *et al.* (1995) A test of the adaptive plasticity hypothesis using transgenic and mutant plants disabled in phytochrome-mediated elongation responses to neighbors. *Am. Nat.* 146, 937–953
- Lai, C. *et al.* (1994) Naturally occurring variation in bristle number and DNA polymorphisms at the scabrous locus of *Drosophila melanogaster*. *Science* 266, 1697–1702
- McColl, G. *et al.* (1996) Response of two heat shock genes to selection for knockdown heat resistance in *Drosophila melanogaster*. *Genetics* 143, 1615–1627
- Siegal, M.L. and Hartl, D.L. (1996) Transgene coplacement and high efficiency site-specific recombination with the Cre/loxP system in *Drosophila*. *Genetics* 144, 715–726
- Miki, B.L. *et al.* (1993) Procedures for introducing foreign DNA into plants. In *Methods in Plant Molecular Biology and Biotechnology* (Glick, B.R. and Thompson, J.E., eds), pp. 67–88, CRC Press
- Weising, K. *et al.* (1988) Foreign genes in plants: transfer, structure, expression, and applications. *Annu. Rev. Genet.* 22, 421–477
- Hansen, G. and Wright, M.S. (1999) Recent advances in the transformation of plants. *Trends Plant Sci.* 4, 226–231
- Macleay, N. (1994) *Animals with Novel Genes*, Cambridge University Press
- Ashburner, M. *et al.* (1998) Prospects for the genetic transformation of arthropods. *Insect Mol. Biol.* 7, 201–213
- Spradling, A.C. and Rubin, G.M. (1982) Transposition of cloned P elements into *Drosophila* germline chromosomes. *Science* 218, 341–353
- Gaugler, R. *et al.* (1997) Field release and environmental fate of a transgenic entomopathic nematode. *Biol. Control* 9, 75–80
- Jaenisch, R. (1988) Transgenic animals. *Science* 240, 1468–1474
- Zambryski, P. (1988) Basic processes underlying *Agrobacterium*-mediated DNA transfer to plant cells. *Annu. Rev. Genet.* 22, 1–30
- Golic, K.G. (1994) Local transposition of P elements in *Drosophila melanogaster* and recombination between duplication elements using site-specific recombinase. *Genetics* 137, 551–563
- Struhl, G. and Basler, K. (1993) Organized activity of wingless protein in *Drosophila*. *Cell* 72, 527–540
- Brand, A.H. and Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118, 401–415
- Mello, C.C. *et al.* (1991) Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* 10, 3959–3970