EVOLUTION & DEVELOPMENT 6:2, 114–122 (2003)

The environmental and genetic regulation of *obake* expressivity: morphogenetic fields as evolvable systems

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SUMMARY The morphogenetic field, a fundamental concept of classical embryology, is once again being invoked to describe developmental processes. Because the evolution of adult structures requires the modification of development, the ways in which morphogenetic fields can change over time may yield insights into evolutionary possibilities. We considered how the duplication/multiplication of a morphogenetic field in fruit flies, caused by the previously described *obake* (*obk*) mutation, is regulated by genetic and environmental factors. Mutations of genes in the canonical antenna-producing imaginal disc pathway suppressed duplication as expected, although the results suggested that other pathways might also be involved. Overgrowth mutations, expected to increase duplication, actually suppressed it. Mutations in the heat-shock protein gene *Hsp83* did not uniformly enhance *obk* expressivity as hypothesized. Using third chromosomes extracted from wild-derived lines, natural genetic variation for modifiers of *obk* function was found to be extensive. Larval crowding suppressed the *obk* phenotype, but there was no evidence of trade-offs between body or head size and arista number. Our results suggest that a complex interplay of genetic and environmental factors in the regulation of fields may be responsible for ample natural variation in the expressivity of adult phenotypes, affording multiple opportunities for selection and evolutionary modification.

INTRODUCTION

The concept of evolvability is currently a topic of much discussion in evolutionary developmental biology. It has been considered both in the context of cryptic genetic variation (reviewed in Waddington 1961) and in terms of "modularity" (Gerhart and Kirschner 1997). One of the early pioneers of the first approach was Waddington, who suggested that hidden genetic variability provided a plausible vehicle for rapid evolution. Waddington demonstrated in fruit flies that the penetrance of the crossveinless phenotype (phenocopy) produced by pupal heat shock could be selected upon. Furthermore, after fewer than 20 generations of selection, the vein loss was found even in the absence of heat shock (Waddington 1953), suggesting that selection had acted on hidden genetic variation for vein formation. More recently, Rutherford and Lindquist (1998) suggested that one mechanism to permit the expression of genetic variability lies in mutations in putative chaperone genes such as those for heat shock proteins. Their finding of increased frequencies of morphological abnormalities in stocks with mutations in the heat-shock protein gene *Hsp83* provides an avenue for exploring particular mechanisms underlying the genetic basis for phenotypic evolvability.

The modularity approach to evolvability considers partially coupled subsystems that can change in evolution (Simon 1973). They are found at all levels of biological organization. These evolvable subsystems (modules) include both molecules such as transcription factors and the transcription cascades of which they are a part. At the subcellular level, mitochondria provide an example of an evolvable partially coupled organelle that is free to evolve as long as the functions essential to cell survival are not impaired. At least two different types of modular evolution can be observed. The first is change within a module (the alteration of the amino acid sequence of a protein being an obvious example). Another type of modular change affects the coupling of subsystems rather than their content. *Hox* genes, with their important roles in body plan morphogenesis in different phyla, are an example of this principle in operation. Indeed, Slack et al. (1993) suggest that *Hox* cluster genes code relative position in animal embryos and that such expression patterns provide a morphological definition of animals. However, despite their conservation, the *Hox* genes regulate the production of different morphologies in each phylum.

Simon (1973) discussed the relationship between evolvability, modularity, and the nature of hierarchical systems. At a

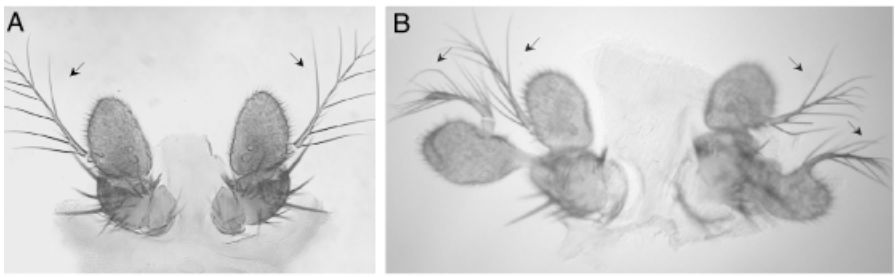

Fig. 1. The *obk* mutation leads to antennal duplications. (A) Wild-type *Drosophila melanogaster* antennae. (B) Antennae of a *w*; *obk* fly. The number of aristae (arrows) was used as a measure of the expressivity of the mutation.

multicellular level of organization, there is an enigmatic developmental module—the morphogenetic field. For the purposes of the present study, a morphogenetic field may be defined operationally as a region of a developing organism with emergent behavior that is generally a property of groups of cells. These regions will develop autonomously if transplanted at the right stage to an ectopic site, and they show regulatory abilities such that a partial field may develop into a complete (albeit smaller) organ. The mechanistic basis of the self-regulation of fields has remained elusive, although a number of mutations have been found that alter fields such that they produce mirror image duplicates or ectopic structures (Clark and Russell 1977; Basler and Struhl 1994; Diaz-Benjumenea et al. 1994; Tabata et al. 1995). Presumably, whether one perturbs a field by physical bifurcation or by manipulating gene expression, the regulatory properties are stimulated by a loss of communication between parts of the field. Properties of morphogenetic fields have been widely studied in insect imaginal discs, as described in the above references, as well as in the presumptive leg tissue in amphibians and chicks (Gilbert 2000).

The potential importance of morphogenetic fields in evolution was brought home to us when we set about "designing" a biramous appendage in the fruit fly consisting of an antenna–leg combination. An important element in the design was a mutation, *obake* (*obk*), which is able to duplicate the antenna-producing morphogenetic field (Dworkin et al. 2001) (Fig. 1). Despite the importance of morphogenetic field behavior in development and its potential as an "evolvable module," it has received little recent attention with respect to either development or evolution (Gilbert et al. 1996).

Here we demonstrate that the duplication processes of the morphogenetic field can be modified by both environmental and genetic variation. Using the *obk* genetic background, we show that larval density alters *obk* expressivity. We explored genetic interaction with *obk* in several different ways. We used the *obk* background to examine the effects of candidate genes known to influence properties of morphogenetic fields in a "sensitized" background. Specifically, we explored the interactions of *obk* and genes such as *wingless* (*wg*) and *decapentaplegic* (*dpp*) that are known to be involved in pattern formation in antenna imaginal discs. Hypomorphic mutations of these genes have been shown to inhibit field duplication in imaginal discs (Buratovich and Bryant 1995). We also explore the interaction of *obk* with a number of genes whose mutations are known to cause hyperplastic growth of imaginal disc tissue. These mutations have been shown to interact synergistically in their ability to produce duplications of the morphogenetic field (Buratovich and Bryant 1997).

Mutations in *Hsp83*, which codes for a putative chaperone (Hsp90), were tested in the presence of *obk* to explore the possibility that they could lead to higher levels of expressivity of the *obk* phenotype. In addition, we found that the genetic background of wild caught flies can influence the expressivity of the *obk* mutation. Our results demonstrate ways in which genes and environment may interact in evolving morphogenetic fields and suggest that further exploration of morphogenetic fields as evolvable modules is both possible and desirable.

MATERIALS AND METHODS

Stocks were raised on a cornmeal/sucrose/yeast medium at room temperature or 25°C, and larvae used for experiments were grown at 25°C. Where necessary, first instar larvae were transferred to vials from apple juice agar plates (4% agar in 50% apple juice, 50% distilled H_2O) on which 20–30 pairs of flies, placed in a plastic beaker inverted over the Petri plate, laid eggs. For tests of *obk* in combination with chromosomes extracted from isofemale lines, the density was set at 30 larvae per vial.

Interaction of *obk* with patterning and overgrowth mutations

Table 1 contains a list of the stocks used in the study, along with the suppliers. The following genotypes were synthesized using standard techniques: *obk* wg^{cx4}/CyO, *obk* wg^{cx3}/CyO,

Table 1. *Drosophila* genotypes used in experiments on the genetic regulation of *obake* (*obk*) expressivity

Allele	Source
obk	S. Tanda, Ohio University
Heat shock protein alleles	H. Lipshitz, University of Toronto
$Hsp83^{19F2}/TM3\ Sb$	
$Hsp83^{08445}/TM3\ Sb$	
$Hsp83^{i5C2}/TM3\ Sb$	
$Hsp83^{P582}/TM3\ Sb$	
$Hsp83^{e6A}/TM3\ Sb$	
$Hsp83^{e6D}/TM3\ Sb$	
Patterning gene mutations	
wg^{1-8}/CyO	Bloomington Stock Center, Indiana University
wg^{cx3}/CyO	
wg^{cx4}/CyO	
dpp^{d5}/CyO	
dpp^{d12}/CyO	
Overgrowth mutations	
$knt^{ri\text{-}1}hyd^{l5}e^{1}/TM3\ Sb\ e$	Bloomington Stock Center
$l(2)ft^{a13}\ or/Cyo$	P. Bryant, University of California, Irvine
$l(2)ft^{fd}\ dp^{ovn}\ or/Cyo$	
$l(2)gd^{d7}/Bc\ Gla\ Elp$	
ex^{br}	

obk wg $^{1\text{-}8}$*/CyO*, *obk* dpp^{d5}/CyO, *obk* dpp^{d12}/CyO, *obk* $l(2)ft^{fd}/CyO$, *obk* $l(2)ft^{a13}/CyO$, *obk* $l(2)gdl^{d7}/CyO$, *obk* ex^{br}/CyO, *obk/CyO;* $hyd^{l5}/TM3\ Sb$. (Note that in addition to the genes of interest, some of the chromosomes in Table 1 contain additional recessive mutations used as markers by the supplying laboratories; these were not tested for in the recombinants.) The synthesized genotypes were crossed to *obk* to yield mutants homozygous for *obk* and heterozygous for one of the patterning or overgrowth mutations. In essence, this is the same method used for genetic screens in "sensitized backgrounds" (Daga and Banerjee 1994; Greenspan 1997; Rutherford 2000).

Flies were grown in vials of an agar cornmeal medium containing molasses. Each cross consisted of two males crossed to two virgin females, and each replicate consisted of three to five crosses made concurrently. The parents were allowed to lay approximately 30 eggs after which they were transferred to another vial. As the first filial generation emerged, they were anesthetized with carbon dioxide and then transferred to Eppendorf tubes containing 70% ethanol. Only the first filial generation was collected. Adults were scored for duplications (or multiplications) of the aristae. The left and right sides were scored separately and summed together to yield the total number of aristae.

We used nonparametric methods of analysis because the data were not normally distributed. In no cases were replicate effects significant after corrections for multiple comparisons. For the interaction of *wg*, *dpp*, and *obk* and for the interactions of *obk* with the overgrowth mutations, we used a Kruskal-Wallis test (Sokal and Rohlf 1995) to examine overall patterns. This was then followed by pairwise Mann Whitney U tests corrected for multiple contrasts using the sequential Bonferroni procedure.

Effect of *Hsp83* alleles on *obk* expressivity

Synthesized genotypes, homozygous for *obk* and heterozygous for an *Hsp83* allele balanced over *TM3 Sb*, were crossed with a homozygous *obk* stock. Up to five vials of each cross were made with five pairs per vial; replicates were made by transferring parents to new vials 2, 3, and 4 days after mating. After 5 days, parents were stored in 70% alcohol. Only female data are reported because males have low expressivity and penetrance (Dworkin et al. 2001). Replicates showed no significant differences and were pooled.

Effects of density

In addition to number of aristae per fly, size measurements were made from images of (female) *obk* flies using a dissecting microscope and KP-D50 digital camera (Hitachi, Tokyo, Japan). Using the ImagePro Plus 4.1.0.9 computer program (Media Cybernetics, Carlsbad, CA, USA), head measurements were made of the distance between left and right vertical setae, whereas the body size measurements were along the midline from the anterior of the thorax to the posterior tip of the scutellum. Two replicates of 35 flies from the 30 and 120 larvae/vial densities were measured as were 15 flies from the 240 larvae/vial treatment. Replicates were pooled because no significant difference was found between them. Correlation analysis was performed using the Tukey-Kramer honestly significant difference test on the JMP 3.2.2 computer program (SAS Institute, Cary, NC, USA).

RESULTS

To evaluate the evolutionary plasticity of morphogenetic fields, we wanted to know how genetic changes might interact with properties of such fields as well as gene–environment interactions. More specifically, we sought to determine whether the *obk* mutation, which duplicates antenna fields, works within the context of canonical genetic pathways with mutations that are known to result in duplications. Our purpose in doing this was to shed light on how many distinct pathways might be available for field modification. The extent to which there exists naturally occurring genetic variation for modifying fields was also considered, as was the effect of density on *obk* expressivity.

Genetic interactions

Does obk function within the same pathways as wg and dpp?

Complementation studies suggest that *obk* may be an allele of *engrailed* (*en*) or that it interacts with it with respect to its function of duplicating the antenna field (Dworkin et al. 2001). If *obk* is indeed a neomorphic allele of *en*, then we can predict that loss of function alleles of other genes that interact with *en* to pattern tissues should lead to a reduction in the number of duplicate fields observed in combination with the *obk* mutant. Alleles of *wg* and *dpp* were combined with homozygous *obk* and tested for changes in penetrance and expressivity. Because *wg* and *dpp* expression are both required for organizing distal outgrowth, it was hypothesized that if *obk* acted in the same pathway, we would expect decreased duplications in the presence of hypomorphic mutations, much as Buratovich and Bryant (1995) found when *wg* and *dpp* mutations suppressed pattern duplications in combination with a mutation in *lethal* (*2*) *giant discs 1*. Furthermore, because wg^{cx4} (also referred to as wg^{1-17}) is a null allele of *wg* and dpp^{d12} produces less gene product than dpp^{d5}, we hypothesized that wg^{cx4} *obk* flies should have fewer duplications than wg^{cx3} *obk* or wg^{1-8} *obk* flies, with similar results for *obk* dpp^{d12} compared with *obk* dpp^{d5}. *obk* dpp^{d12} wg^{+}/*obk* dpp^{+} wg^{cx4} double heterozygous flies were expected to have the fewest duplications.

In Fig. 2, we present data for female flies because their increased sensitivity to *obk* provided a greater range of potential suppressive effects, but similar results are found with males. Consistent with the hypothesis, *obk* in combination with dpp^{d12} did significantly suppress duplications compared with *obk* alone, whereas the weaker allele dpp^{d5} did not. *obk* in combination with the wg^{cx4} and wg^{cx3} alleles also suppressed duplications, although it is surprising that wg^{1-8} did not. Unexpectedly, the double heterozygote, which was expected to be more suppressive than any of the single allele combinations, did suppress *obk* but not significantly more than the single combination strains with *wg* or *dpp*. The explanation for these findings is unclear. One possible explanation is that the function of *wg* and *dpp* are only partially coupled to the duplicating effects of *obk*. On balance, the data suggest that canonical patterning genes may be involved in *obk*-induced antenna duplication.

Does obk interact with overgrowth mutations?

Overgrowth (hyperplastic) mutations are a useful class of mutations for investigating morphogenetic fields because of the possibility that patterning defects or duplications occur when proliferation causes breakdowns in communication between different parts of the field. Our tests involved alleles of *fat* (ft^{fd}, ft^{a13}), *hyperplastic discs* (hyd^{l5}), *lethal(2)giant discs 1*, ($l(2)gd1^{d7}$), and *expanded* (ex^{br}). ex^{br}, in addition to increasing the number of cells in the wing (Boedigheimer and Laughon 1993), occasionally leads to duplicate antennae, though the phenotypes tend not to resemble *obk*.

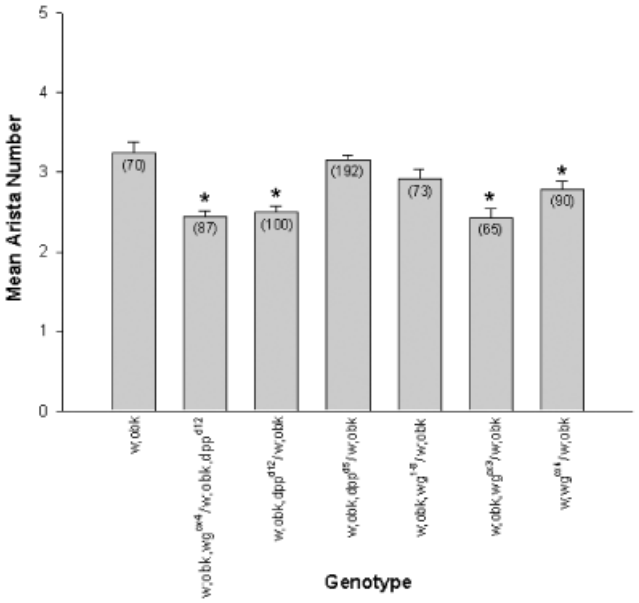

Fig. 2. Comparisons of female mean arista number in a strain of *obk* and strains of *obk* and mutations of *wg* and *dpp*. Asterisk signifies that arista numbers are significantly different from those of the *obk* strain at $\alpha = 0.05$ based on pairwise Mann Whitney U tests corrected for multiple comparisons using the sequential Bonferroni technique. Replicates were pooled. Numbers in parentheses represent number of flies scored. T shows the upper bound of the standard error of the mean.

Figure 3 provides comparisons of mean number of aristae of *obk* females with those of five lines containing heterozygous, hypomorphic, overgrowth alleles combined with *obk*. If *obk* interacted synergistically with the overgrowth genes, we would expect that the combination of *obk* with additional mutations in the pathway would increase the number of antenna duplications. In all comparisons, *obk* arista numbers are significantly higher than those of *obk*–overgrowth mutant combinations, suggesting that their mechanisms of action are not additive or positively synergistic but rather antagonistic with respect to the effects of the *obk* mutation.

Do Hsp83 mutants increase obk penetrance or expressivity?

Rutherford and Lindquist (1998) provided data suggesting that flies with mutations within the putative chaperone gene, *Hsp83*, are less able to buffer ordinarily hidden genetic variation and therefore deleterious mutations within lines will express phenotypic anomalies. Recently, Sollars et al. (2003) suggested that *Hsp83* mutants may act by altering chromatin states rather than as protein chaperones. Both groups hypothesize that *Hsp83* acts as a "capacitor" for morphological evolution. We wanted to know whether mutations in chaperones would not only reveal hidden variability but

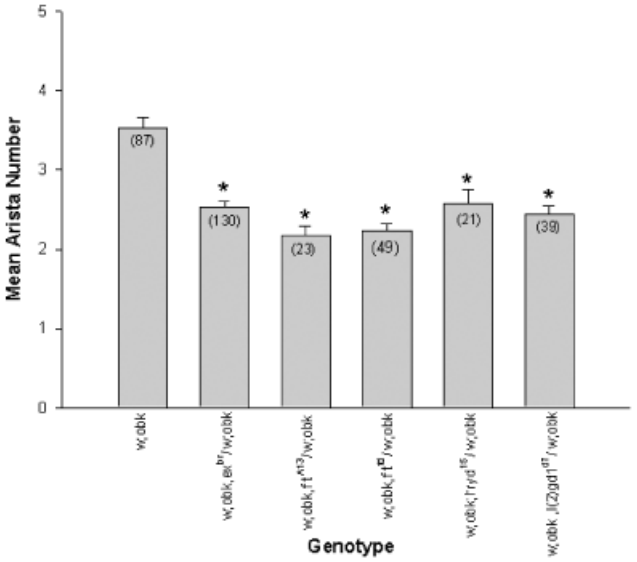

Fig. 3. Comparisons of female mean arista number of a strain of *obk* and strains of *obk* and overgrowth mutations. Asterisk signifies that arista numbers are significantly different from those of the *obk* strain at $\alpha = 0.05$ based on pairwise Mann Whitney U tests corrected for multiple comparisons using the sequential Bonferroni technique. Replicates were pooled. Numbers in parentheses represent number of flies scored. T shows the upper bound of the standard error of the mean.

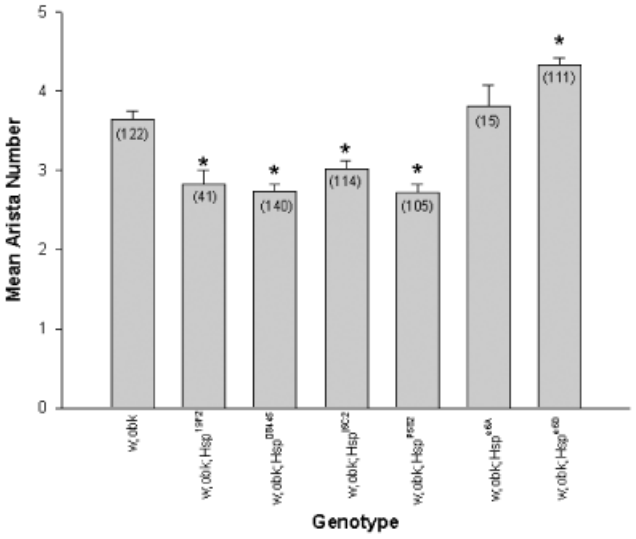

Fig. 4. Comparisons of female mean arista number in a strain of *obk* and strains of *obk* and *Hsp83* mutations. Asterisk signifies that arista numbers are significantly different from those of the *obk* strain at $\alpha = 0.05$ based on the Tukey-Kramer HSD test. Replicates were pooled. Numbers in parentheses represent numbers of flies scored. T shows the upper bound of the standard error of the mean.

perhaps also increase the penetrance and expressivity of mutations such as *obk*. The importance for us lies in the problem that such a mutation, which is highly variable in penetrance and expressivity, would need to be stabilized for it to become a fixed part of the population phenotype.

We combined *obk* with *Hsp83* alleles studied by Rutherford and Lindquist (1998), including the allele Hsp^{P582}, which is a null (Basirullah and Lipshitz, personal communication). Because these alleles are usually homozygous lethal, we balanced them over a *TM3 Sb* chromosome. We observed that the *TM3* chromosome suppressed the *obk* phenotype (data not shown), so in our tests we crossed *obk*;*Hsp83* mutant/Balancer to *w*;*obk*; +/+ and scored only those without the balancer chromosome.

In Fig. 4 we show the female data of strains combining *obk* with six different *Hsp83* mutant alleles. Only two alleles, $Hsp83^{e6a}$ and $Hsp83^{e6d}$, had higher expressivity (number of aristae) than the laboratory *obk* stock, and these alleles were obtained in the same mutant screen. This fact, and the fact that Hsp^{P582}, the null allele, did not enhance expressivity, suggests that the effects we are seeing may be due to genetic background and not specifically to *Hsp83* mutations.

Does natural variation affect obk expression?

To determine whether natural genetic variation could influence the expressivity of *obk*, we combined *obk* with third chromosomes from isofemale lines obtained from flies caught in Toronto and Algonquin Park (locales in Ontario, Canada, approximately 250 km apart). We used balancer chromosome techniques to extract single third chromosomes that were then combined with the common *obk* stock second chromosome. We began with approximately 30 isofemale lines and in the end tested eight viable lines that were homozygous for the *obk* second chromosome and a third chromosome derived from an isofemale line. Figure 5A shows the means and standard error of the means of the eight lines. A significant line term was found in an analysis of variance ($P < 0.00001$) consistent with the interpretation of natural genetic variation affecting *obk* expressivity.

We chose a low line from the Toronto area and a high line from each of the two locales and crossed low by high and high by high lines (Fig. 5B). The high line by high line crosses produce an F1 with high expressivity (measured as total number of aristae per fly), whereas the high by low crosses produced lines with intermediate expressivity. Thus, there exists naturally occurring genetic variability to both enhance and suppress *obk* expressivity. The high lines may share identical, additive, or codominant alleles given that their F1 is as susceptible to antenna duplication as either parent. The intermediate values for antenna duplication seen in the cross of either high line to the low line is open to a number of genetic explanations but seems to rule out the simplest one of a single dominant allele conferring high or low duplication propensities.

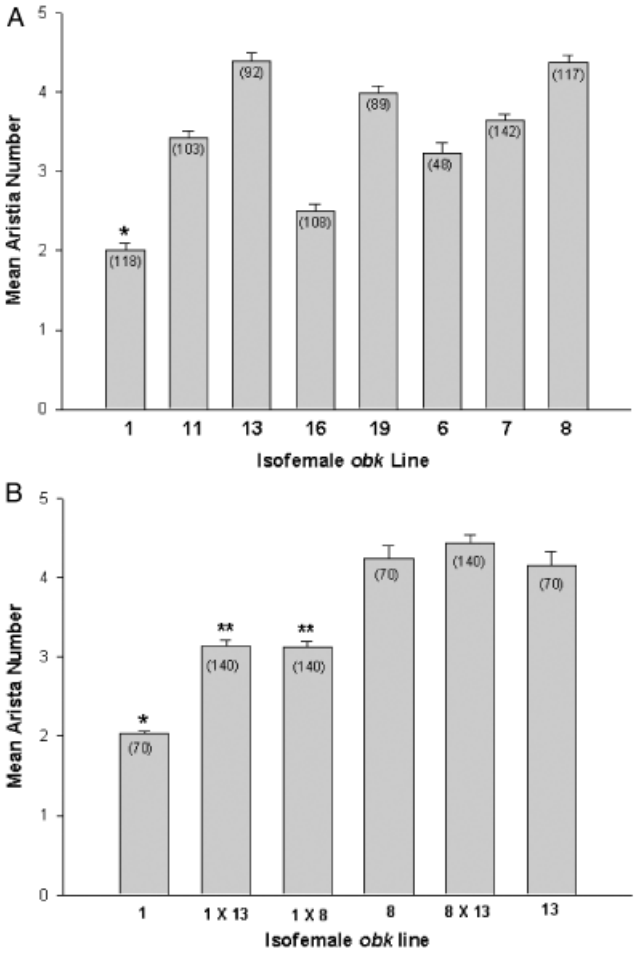

Fig. 5. (A) Comparisons of mean arista number of females in eight lines with *obk* on the second chromosome and a third chromosome derived from an isofemale line. Asterisk indicates significant difference from the rest using the Tukey-Kramer HSD test. (B) Comparisons of mean arista number of females in three of the lines in (4A) and their F1 offspring. *Significantly different from the rest, **significantly different from isofemale *obk* lines 8, 13, and 8X13 using the Tukey-Kramer HSD test. Replicates are pooled and parentheses enclose the number of flies scored. T shows the upper bound of the standard error of the mean.

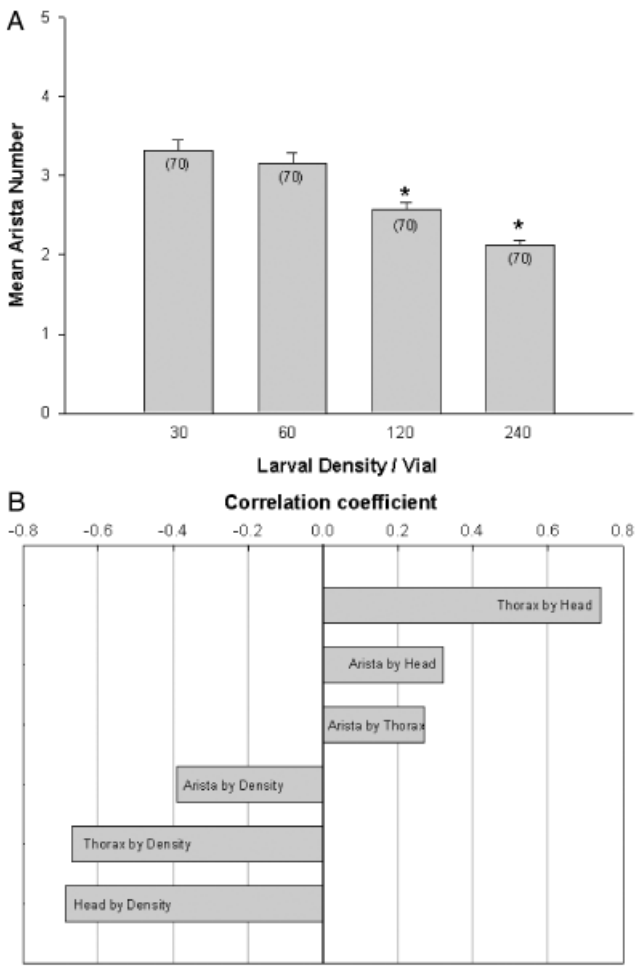

Fig. 6. (A) The effect of larval density on female arista numbers in *w;obk* flies. *Significantly different from the rest using the Tukey-Kramer HSD test. Replicates are pooled and the number of flies scored are in parentheses. T shows the upper bound of the standard error of the mean. (B) Correlations between arista number, head and thorax size, and larval density. All correlations shown are significant ($P<0.01$).

Environmental effects on *obk* expression

Is obk expressivity reduced with increased larval density?

Casual observation had suggested that *obk* penetrance and expressivity is reduced in old crowded cultures. For this reason, we were careful to control for density in our experiments. Because of our interest in environment–gene interactions and the possibility of studying allometric consequences of an environmental effect, we quantified the effect of larval density on *obk* expressivity in females. Replicated treatments of 30, 60, 120, and 240 larvae per vial were analyzed and no significant difference between replicates was found. Above 60 larvae per vial, the mean number of aristae decreased significantly, as seen in Fig. 6A. Survival was 80% or higher for all treatments with the exception of 240 larvae per vial, in which there was less than 60% survival. (In the latter case, the crowded conditions and viscous media sometimes led to accidental drowning.) We then measured heads and thoraces of flies from treatments of 30, 120, and 240 larvae per vial, looking for correlations between arista numbers, head and body measurements, and larval density (Fig. 6B). Surprisingly, although the three measured traits decrease as density increases, arista numbers are positively correlated with head and thorax measurements. This indicates that only the larger flies increased antenna numbers; we saw no evidence for trade-offs between body/head measurements and number of antennae.

DISCUSSION

Are different morphogenetic fields formed by common molecular mechanisms?

One of the intriguing aspects of morphogenetic fields is that they are defined operationally (by what they do, not by their composition or their mechanistic basis) and that the properties so defined are useful in describing the development of a variety of structures in diverse taxa (Gilbert et al. 1996). It is tempting to suggest that not only are the "rules" of morphogenetic fields similar but that their material basis, the molecules involved, are universal as well. One way of testing the potential molecular universality of morphogenetic fields is to test for interactions among mutations that perturb fields. Epistatic interactions would suggest that similar pathways are affected by the mutations, lending support to the hypothesis that there is a conserved morphogenetic field pathway. Wg and Dpp function in imaginal disc fields as well as in other structures in developing flies. They are important players in signal transduction in imaginal discs and their co-occurrence establishes the point of distal outgrowth of appendages (Cohen 1993). Hypomorphic mutations in the genes encoding these proteins have reduced the frequency of pattern duplications in $l(2)gd1^{1}$ imaginal discs (Buratovich and Bryant 1995). Could they be candidates for defining the morphogenetic field at the molecular level? Although similar molecules have been found in several animal taxa, to our knowledge they have not been identified in plant development, which nevertheless has tissues with the field properties of polarity, gradients, and regulation (Sachs 1991), or in unicellular ciliates, which also demonstrate field phenomena (Frankel 1997). The overgrowth class of mutations perturb animal morphogenetic fields, presumably because growth itself can separate regions that previously had communicated by paracrine signaling operating over distances of only a few cell diameters (Larsen 2003).

We studied the interactions of *obk* with *wg* and *dpp*. Our results indicate that *wg* and *dpp* mutations interact with *obk*, but the quantitative differences we expected between single and double heterozygotes were not realized. These data suggest that *obk* requires some of the canonical molecules for fly appendage development to produce antenna duplications, but we suspect that other yet to be identified pathways are also involved.

Although *obk* shows similarities with some mutations identified as overgrowth mutations (by virtue of its pattern duplication effects and the enlarged size of the duplicated structures), homozygous *obk* does not seem to interact synergistically with single copies of a duplicating allele of *l(2)gd1* or two alleles of *l(2)ft*. In fact, contrary to our expectations, the overgrowth mutations appear to have an antagonistic effect on the duplicating properties of the *obk* mutation. In contrast to our results, Buratovich and Bryant (1997) found that loss of one copy of *l(2)ft* synergistically interacted with homozygous *l(2)gd1* to produce antenna disc duplications. Conversely, $l(2)gd1^{d7}$ displays a dominant synergism with homozygous $l(2)ft^{fd}$ to produce antenna disc duplications. All the overgrowth mutants we tested with homozygous *obk* resulted in statistically significant suppression of antenna duplication. Thus, whether or not animal morphogenetic fields are produced by the same molecular mechanisms, our results indicate there may be many routes by which they may be perturbed. Although this makes the situation more complex from a molecular developmental perspective, from an evolutionary biology perspective it provides more avenues for modification.

Is there natural genetic variation that can modulate morphogenetic fields?

We were surprised at both the enhancing and suppressive effects of natural genetic variation modulating antenna duplication uncovered in the eight strains in which isogenic third chromosomes were combined with *obk*. These results may be relevant to the hopeful monster hypothesis (Goldschmidt 1933), which asserts that mutations of large effect can play a role in the evolution of form. If a mutation like *obk* arose in a genetic background that was suppressive, it could increase in frequency, producing homozygotes from time to time, until such a time as it was expressed in a nonsuppressive genetic background or in a different environment (in the example of *obk*, that new environment might be less resource limiting). The consequence of either of these changes would be to increase the likelihood that if the mutation's phenotype were adaptive, it might well meet a cohort of monsters with which to breed. A likely scenario? Perhaps not, but then evolution is the result of unlikely events, and such a possibility should not be dismissed out of hand because we fail to consider the role that the elusive "genetic background" plays in determining the development of phenotype (Wilkins 2002, pp. 350–352; Dworkin et al. 2003). In fact, even mutations of essential genes like *Ubx*, widely used in arthropod development for segment identification and modulation, may respond to genetic background (Larsen 1989; Gibson and van Helden 1997). Indeed, this should be the expectation if most "naturally occurring" mutations, whether in nature or the laboratory, are the result of insertions of transposable elements. Such a scenario is not accounted for in the classic Fisher model of evolution by infinitesimally small steps. However, Orr (1998) derived a more realistic distribution of the relative sizes of adaptive mutations, showing that a few loci of large effect may account for a considerable proportion of phenotypic variation. This interpretation of evolution is supported empirically by quantitative trait locus analysis in a number of organisms (Mackay 1995; Bradshaw et al. 1998).

Loss of function of *Hsp83* has no effect on *obk* expressivity

Because we were interested in the architecture of genetic variation affecting *obk*, we were curious as to whether or not loss of function of a chaperone such as *Hsp83* (which is implicated as part of a general buffering mechanism) would reveal additional genetic variation. Given that we observed no effects due to loss of function of the heat shock protein, we can consider several explanations.

First, *Hsp83* functions as part of a buffering mechanism that may not be so general as to include arista number as studied in this experiment. A second possible explanation is that the *obk* mutation already removes the antenna field from the "zone of canalization" where buffering of natural genetic variation occurs. Thus, all the cryptic genetic variation for aristae has already been revealed; therefore, loss of buffering in the *Hsp83* mutant background would have no additional effect. Finally, it is possible that the effects of genetic background may be strong enough to override the influence of loss of *Hsp90* on *obk* penetrance. This scenario is reinforced by the finding that the only two *Hsp83* mutations that did not significantly reduce arista number when combined with *obk* were from the same screen.

Are there trade-offs with the size of other body parts when one structure overgrows?

When a structure grows larger than normal or is replaced by a larger structure, it is reasonable to ask where the cells come from. One can imagine three scenarios: (a) there are no new cells, only larger ones; (b) there is increased cell proliferation; or (c) cells that would ordinarily be slated for one structure are co-opted for another (Nijhout and Emlen 1998).

Because a casual examination of nuclear density in imaginal discs does not suggest an increase in cell size, we suspected that some combination of cell proliferation and co-option would occur. Evidence that co-option occurs in nature is seen in the work of Emlen (2001), who described the size trade-offs of eyes versus horns in a sexually selected trait in the dung beetle, *Onthophagus*. Our correlation of head and body size to number of aristae was slightly positive, inconsistent with the trade-off model. In an interesting union of ecology and developmental mechanism, several possibilities might be tested. One is that co-option occurs when the gene expression responsible for the enlarged structure ventures into the domain of a nearby structure, whereas co-option is less likely when the "aberrant" gene expression is confined to only one morphogenetic field. Another possibility is that the trade-offs in the case of the beetle horns may have evolved after the production of horns proved adaptively advantageous. Presumably, in the case of *obk*, one could select for a trade-off with body size and/or head size. Finally, trade-offs may be occurring to some extent even in the case of *obk* but might be very local and not seen in gross head dimensions.

Evolutionary implications of morphogenetic field regulation

Despite the usefulness of the morphogenetic field concept for unifying a large number of developmental phenomena, little attention has been given to the role in evolution played by alterations in the genetic control of fields. One of the properties of fields is that they change dynamically during development, their properties varying with time. Changing the rate of development of a field may have interesting consequences. For example, in opossums, the forelegs of the embryo mature relatively early, allowing the immature pup to crawl into its mother's pouch at a time when the hind legs are not visibly present. One might profitably look at regeneration of appendages as a reforming of a morphogenetic field at a wound site and explore whether this is the result of calling up a preexisting developmental module during adulthood. Conversely, the loss of some structures in evolution may be investigated from the point of view of suppressing morphogenetic fields, whereas the evolution of new structures like insect wings may be investigated as the emancipation of a new field from an existing field. If the morphogenetic fields concept can help us understand insect wing evolution in a six-legged taxon, perhaps we will be able to consider why Pegasus is still a creature of our imagination.

Acknowledgments

This work was supported by an NSERC grant to E. L., a postgraduate NSERC fellowship to I. D., and an Ontario Graduate Fellowship to J. A. We gratefully acknowledge the *obk* strains received from S. Tanda. We are also grateful for technical assistance provided by Tran Le, Anna Leung, Yuli Raharja, and Chermain Yiu.

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