

## *Drosophila* mutants suggest a strong drive toward complexity in evolution

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**SUMMARY** The view that complexity increases in evolution is uncontroversial, yet little is known about the possible causes of such a trend. One hypothesis, the Zero Force Evolutionary Law (ZFEL), predicts a strong drive toward complexity, although such a tendency can be overwhelmed by selection and constraints. In the absence of strong opposition, heritable variation accumulates and complexity increases. In order to investigate this claim, we evaluate the gross morphological complexity of laboratory mutants in *Drosophila melanogaster*, which represent organisms that arise in a context where selective forces are greatly reduced. Complexi-

ty was measured with respect to part types, shape, and color over two independent focal levels. Compared to the wild type, we find that *D. melanogaster* mutants are significantly more complex. When the parts of mutants are categorized by degree of constraint, we find that weakly constrained parts are significantly more complex than more constrained parts. These results support the ZFEL hypothesis. They also represent a first step in establishing the domain of application of the ZFEL and show one way in which a larger empirical investigation of the principle might proceed.

The view that complexity increases in evolution has been of interest in evolutionary studies since Darwin. What began as impressionistic assessments of the history of life, based on the fossil record (Lamarck 1809 [1984]; Darwin 1839 [1987]; Spencer 1863; Cope 1871; Huxley 1953), developed into empirical and conceptual work on trends—specifically, the trend toward complexity—and trend mechanisms in evolution (Rensch 1960; Simpson 1961; Schopf et al. 1975; Saunders and Ho 1976; Papentin 1980; Maze and Scagel 1983; Wicken 1987; Brooks and Wiley 1988; Valentine et al. 1994; McShea 1996; Bonner 1998; Saunders et al. 1999; Stoltzfus 1999; Gray et al. 2010). A persistent problem throughout this research has been the problem of how to operationalize complexity. Darwin's suggestion to use “differentiation of parts and consequent complexity of structure, as the standard of comparison” in orchids (Darwin 1862, p. 333) was prescient. And indeed the most commonly adopted operationalization in use today is the amount of variation among the component units, or parts, of an organism, whether the parts are genes, molecules, cells, or larger-scale anatomical units (e.g., Saunders et al. 1999; Sidor 2001; Buchholtz and Wolkovich 2005; Marcus 2005; Adamowicz et al. 2008). In other words, complexity is a variance measure, with variance understood in a continuous sense, as the amount of differentiation among parts, or—going beyond Darwin—in a discrete sense, as the number of different part types (Valentine et al. 1994).

Most of these studies take for granted that a trend in complexity occurred (cf. McShea 1991), but the mechanism

behind the trend—the cause—is controversial. One question in particular has been whether or not this trend is the result of selection. Bonner (1988) argued that complexity arises as a result of the advantages of differentiation of parts and division of labor, especially in large organisms. (See McShea 1991, 1996, for a discussion of other selection-driven mechanisms.) McShea and Brandon (2010), on the other hand, present an alternative view that differentiation of parts is expected to increase spontaneously, in the absence of selection. The latter hypothesis, called the Zero Force Evolutionary Law, or ZFEL (McShea and Brandon 2010), predicts a strong drive toward complexity, a tendency for complexity to increase that is present in all lineages, and at all times, in the history of life. As they point out, this tendency will not always be realized, because it can be (and often will be) overcome by selection and/or constraints. But when opposition is absent, the ZFEL argument goes, heritable variation accumulates and parts will tend to become different from each other, in other words, complexity—understood as differentiation among parts or number of part types—increases. According to the McShea and Brandon argument, a ZFEL-driven increase in complexity can result from drift, that is, from parts randomly changing. But it can also result from selection acting to some degree independently on different parts, for example, selection on each part to specialize for a different function. (Selection is not completely absent in this case, obviously; rather, it is absent in the sense that there is no selection for complexity per se; see McShea and Brandon 2010 for further discussion.)

The main idea behind the ZFEL—as variation accumulates, variances tend to increase—is not new, and the logic is present implicitly or explicitly in a variety of contexts in evolutionary theory. For example, the process of duplication and differentiation of parts, identified more than three quarters of a century ago by vertebrate morphologists (e.g., Gregory 1934, 1935) is the ZFEL. So is the tendency for left–right asymmetry to rise in bilaterians in the absence of developmental buffering, or fluctuating asymmetry (Van Valen 1962). The ZFEL is implicit in standard phylogenetic models that treat species as particles and the evolution of their characters as a Markov process in a morphology state space (Raup et al. 1973). Finally, the ZFEL is implicit in recent work on gene duplication and differentiation (Taylor and Raes 2004; Lynch 2007), and on molecular differentiation more generally (Finnigan et al. 2012). The ZFEL hypothesis unifies all these theories in that it applies at all levels of the biological hierarchy within organisms—molecules, organelles, cells, tissues, organs, organ systems, and all levels in between. As we discuss later, it applies independently at each level as well. (And perhaps obviously, the ZFEL also applies at all higher levels—organisms, populations, species, and clades—although usually the term diversity is used, rather than complexity, to describe differentiation at these higher levels.)

Here we investigate the ZFEL hypothesis by evaluating the gross morphological complexity of laboratory mutants in *Drosophila melanogaster* and comparing them to the complexity of wild-type flies. Laboratory mutants represent organisms that arise in a context where selective forces are greatly reduced. Organisms in the wild contend with disease, predation, resource limitation, and environmental disturbances, among other things; however, this “ecological component” of selection is greatly reduced when organisms are artificially supported in a laboratory setting, as in the case of *D. melanogaster* mutants. Thus, the prediction is that mutants will on average be more complex than the wild type. Here we compare the complexity of gross morphology in *D. melanogaster* mutants to that of the wild type using three measures—part-type count, shape, and color—and apply these measures to part types at two focal levels.

It may be helpful at this point to say something about the relationship between the mechanism underlying the ZFEL and proposed mechanisms of evolutionary change based on entropy (Pringle 1951; Wicken 1987; Brooks and Wiley 1988). Both invoke the spontaneous tendency for points in a space to disperse, in this case points in a morphospace, with points representing parts and their dispersion representing an increase in the differences among them (differentiation among parts) or in the number of discrete regions of the space occupied (number of part types). The similarity is real, but for discussions of complexity in the sense in which we use it here, entropy is a misleading term. The main reason is that in one standard interpretation of entropy, there is no level independence. In this interpretation, entropy cannot simultaneously increase at the level of the whole while decreasing in every one of its parts. For

example, an evolving organism cannot become more entropic while every one of its cells becomes less. However, the concept of complexity is not constrained in this way. And in fact, McShea (2002) found that in the transition from unicellularity to multicellularity in animals, the number of cell types (i.e., cell-level complexity) increased, while the average number of parts within those cells (i.e., cell-organelle-level complexity) decreased. At a higher level, at least in principle, an organism with many organs need not have many cell types. At a lower level, an organism with many cell types need not have many gene types. The point is that entropy (in one standard interpretation) and complexity (as understood here) are not the same concept. For further discussion, see McShea and Brandon (2010).

## METHODS

Complexity can be measured as the standard deviation—or virtually any other measure of variance—among parts in some dimension. For example, McShea (1993) measured complexity continuously as the amount of differentiation among the vertebral elements in the mammalian vertebral column, using a univariate variance analogue—the average absolute difference between each vertebra and the mean—for each vertebral dimension measured. We used a discrete measure of complexity, a count of number of different part types, analogous to Valentine et al.’s (1994) count of cell types in their study comparing cell-type counts among metazoans. Unlike Valentine et al., we looked at large-scale structures, and we also looked at two levels of organization, what we call part and subpart. We treat these levels as independent, because as discussed, complexity values are in principle independent across levels. Of course, despite this in-principle independence, it could be that complexity changes are in fact correlated across levels, but that is something to be discovered empirically, not assumed beforehand. For now, at least, in the absence of any knowledge of the existence of a correlation, testing at two different focal levels will be considered two independent tests of the ZFEL hypothesis.

The dataset used also contained information on color and shape changes in various parts of the mutants, and since the ZFEL applies to any variable, any dimension, in which variation occurs, we took advantage of the opportunity to evaluate complexity differences in these dimensions as well.

## PART-TYPE COMPLEXITY

A part is defined as an entity with internal integration and external isolation (McShea and Venit 2001). An eye is a part of a fly because the components that make up the eye are integrated, and the entire eye is identifiably isolated to some degree from the other parts of the fly. Parts can have subparts, for example, an eye has facets and a leg has tarsal segments. Complexity is not a

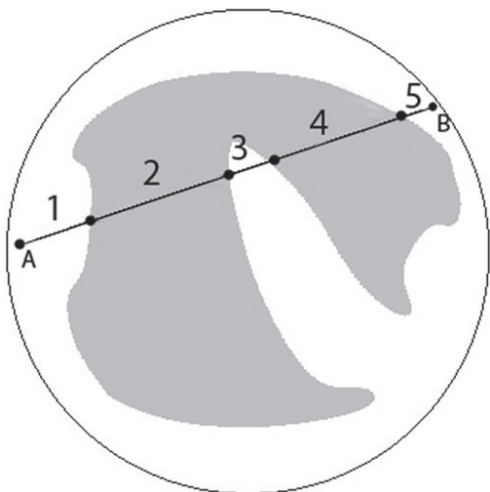
measure of the total number of parts, but of the total number of part types. A part type is unique and the number of instances of a part type does not affect the complexity measure. For example, a wild-type fly has two eyes, however, there is only one eye part type, regardless of the fact that there are two instances of that part type. Similarly, with the ocelli there is just the one ocellus part type even though there are three ocelli. (For further discussion, see McShea and Venit 2001, especially Fig. 1.)

In this study, part types can change in three ways; they can be gained, lost, or replaced. The addition of a new part type is an increase in complexity, the loss of a part type is a decrease in complexity and the replacement of one part type by another type is recorded as no change in complexity. When counting part types, there are six basic routes to those three types of changes. This is shown in Table 1 where the original part type is called “X” and the two different and new part types are called “Y” and “Z.” As an example, in *D. melanogaster*, there are two identical wings, meaning, there are two instances of what we might call the *wing1* part type (X). If both wings remain unchanged, there is no change in complexity. If both wings change in the same way, there is a gain of a *wing2* part type (Y), however, the original *wing1* part type is lost so the number of total part types has not changed and there is no change in complexity. If one of the wings changes to *wing2* and the other does not, or if both wings change in different ways such that there is now a *wing2* and a *wing3* part type (Z), this is an increase in complexity. (In the second case, there is a gain of two types, *wing2* and *wing3*, combined with a loss of one type, *wing1*. Thus, in both cases, there is a net increase of one.) If a third wing arises that is the same as the

**Table 1. Summary of the three different measures of complexity—part-type, shape, color—and the different ways complexity can increase, decrease, or stay the same. “X” represents the original part type and “Y” and “Z” represent new and different part types**

Complexity measure	Complexity change	Description
Any	No change	All Xs stay Xs
Part-type	No change	All Xs become Ys
Part-type	Increase	Some Xs become Ys, some Xs stay Xs
Part-type	Increase	Some Xs become Ys, some Xs become Zs
Part-type	No change	All Xs stay Xs and another X arises
Part-type	Increase	All Xs stay Xs and a Y arises
Part-type	Decrease	All Xs are lost
Shape	Increase	Y is less spherical than X
Shape	Decrease	Y is more spherical than X
Shape	No change	Y is not significantly more or less spherical than X
Color	Increase	Y has more colors than X
Color	Decrease	Y has fewer colors than X
Color	No change	Y does not have more or fewer colors than X

*wing1* part type, there is no change in complexity, but if that third wing is a *wing2* part type, there is an increase in complexity. Lastly, if both wings are lost (i.e., the *wing1* part type is lost), this is a decrease in complexity because the total number of part types has decreased.



**Fig. 1.** To calculate the complexity of an object, encase it within a sphere and pick two random points (A and B) on that sphere to create a global line. When a global line intersects the object, a chord is created. In this illustration, modified from Rigau et al. (2005), five random chords are created. The least complex object would be a sphere because no chords would be generated. The gray object shown is considerably more complex on account of its various concave surface elements.

**SHAPE COMPLEXITY**

In this study, shape is understood in a way that enables us to think about it as a number of potentially differentiated units, or shape segments. The approach is based on Rigau et al. (2005), who apply the following protocol, illustrated in Figure 1. The contour or surface of an object is measured using global lines. An object is encased in an imaginary sphere and on the surface of the sphere two random points are selected to create a global line crossing the object. If these lines intersect the object, random chords are created (see Fig. 1). In our terms, complexity is a function of the number of those chords (for details, see Rigau et al. 2005). Using this function, the least complex shape is a sphere because global lines are possible from any two points without creating any random chords. The most complex shapes have concave surfaces, like a saddle, or distant extensions, like the corners of a tetrahedron. For Rigau et al. (2005), what this approach measures is “how ‘distant’ is any object from the sphere” (p. 360). In our terms, these concave surfaces, saddles, and distant extensions are shape elements, and objects with more

shape-element types are more complex than objects with fewer. Our assumption is that departure from a sphere increases with number of shape-element types.

The Rigau et al. method for calculating shape complexity would be difficult to apply in a quantitative way without detailed morphometric information. Thus, we adopt their approach only in principle and operationalize it by making the following simplifying assumption: Part type Y is more complex in shape than part type X if Y is less sphere-like because of concave (or corner) regions. A less obvious example is an increase in texture, which is an increase in concave (or corner) regions at a smaller scale. As Table 1 shows, more concavity is an increase in shape complexity and becoming more spherical is a decrease in shape complexity. Notice that in this approach, a part type can change shape without a significant increase or decrease in concavity, that is, with no change in shape complexity. As an example, some *D. melanogaster* mutants have bristles that are “reduced” or “smaller” meaning the cylindrical shape of the bristle loses length and possibly loses some width. Bristles have changed shape compared to the wild type, but there is no significant complexity change, just a replacement of the original shape.

## COLOR COMPLEXITY

Here, color complexity is a function of pigmentation, that is, the color complexity of a part depends on a set of pigment subparts. A part that is the least complex in color is completely transparent and contains no pigment. If that same part gains a color (either entirely or selectively in the form of a pattern), it now contains one pigment part type, which represents an increase in color complexity. If another pigment part type is added, such that there are now two colors (or two shades of one color), this is a further increase in color complexity because there are now two different pigment part types. Thus, in assessing color complexity, our protocol is to ask whether a part has gained or lost any color, that is, pigment part types.

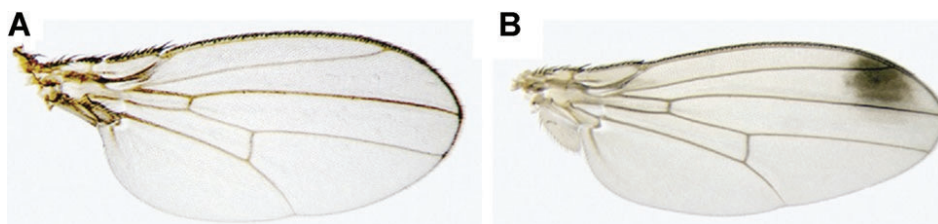
An example is the *D. melanogaster* wild-type wing, which is transparent. A gain in complexity is characterized by an increase in color, i.e., in pigment part types (see Table 1). If we apply this

pigmentation measure to Figure 2, which compares the typical wing type in (a) *D. melanogaster* with (b) *Drosophila suzukii*, the latter is more complex because the dark pigmented spot means it contains one pigment part type while *D. melanogaster* contains none. If a wing of a different species had a similar spot that happened to be two colors, or had two spots of different colors, this would be more complex than (b) because it would contain two pigment part types.

As Table 1 shows, the addition of a color is an addition of a pigment part type and an increase in color complexity. The loss of a color is a loss of a pigment part type and a decrease in color complexity. If a part changes in color but not in number of pigment part types, then the color complexity stays the same; that is, if a part were to change from red to blue, or a spot that was gray turns orange, the color complexity does not change.

## DATA COLLECTION

In this project, the gross morphological complexity of *D. melanogaster* mutants was compared to the *D. melanogaster* wild type. A total of 1955 mutants were analyzed using *The Genome of Drosophila Melanogaster* by Lindsley and Zimm (1992) and the complementary website, <http://flybase.org>. The descriptions and—when available—illustrations were examined, and then the morphological level of each mutational change and its corresponding part-type, shape and/or color complexity, relative to the wild type, was assessed. Two levels of gross morphology were considered: (a) part and (b) subpart. The part level includes the large structures of the entire fly such as the abdomen, antennae, eyes, head, legs, thorax, and wings. The subpart level contains smaller-scale structures lying within the parts, including abdominal segments, antennal segments, eye facets, ocelli, leg segments (joints and divisions), thoracic segments, wing parts (e.g., cells, veins, margins), bristles, and hairs (see Table 2). These gross distinctions were used as a guide for comparing fly descriptions that were usually much more detailed. For example, anatomical parts such as the scutellum, humerus, and haltere all fit under the category of “thoracic segments” in Table 2, and are counted at the subpart level. As



**Fig. 2.** Differences in wing pigmentation between two different species of *Drosophila* to illustrate variation in color complexity: (A) *Drosophila melanogaster* and (B) *Drosophila suzukii*. When measuring color complexity based on pigment part types, *D. melanogaster* (A) is the least complex because it is transparent and lacks any pigment part types. *D. suzukii* is more complex because it contains one pigment part type. Figure modified from Parchem et al. (2007).



**Table 2. The general guideline for categorizing the morphological characteristics of *Drosophila melanogaster* as parts or subparts**

Part	Subpart
Abdomen	Abdominal segments
Antennae	Antennal segments
Eyes	Eye facets
Head	Ocelli
Legs	Leg segments (joints and divisions)
Thorax	Thoracic segments
Wings	Wing parts (e.g., cells, veins, margins) Bristles and hairs

Table was used to identify two independent levels of hierarchy when analyzing the complexity of *D. melanogaster* mutants based on gross morphology.

another example, bristles are often identified by their location such as scutellars, supra-alars, sternopleurals, chaetae (micro and macro), setae, vibrissae, and trichomes. These are all counted at the subpart level. “TermLink” ([http://flybase.org/static\\_pages/termlink/termlink.html](http://flybase.org/static_pages/termlink/termlink.html)) was consulted if there were any terminological questions that arose from a specific description. This resource on <http://flybase.org> contains an extensive list of *Drosophila* anatomy terms, their definitions, their illustrations (if available), their hierarchy within the organism, their relation to

other parts, synonyms for the term, and any literature that might reference the term. Table 3 presents some examples of complexity changes in 11 different *D. melanogaster* mutants (the descriptions are simplified and condensed for the sake of demonstration). Illustrations corresponding to six of the example mutants in Table 3 are presented in Figure 3 alongside illustrations of the wild type.

## DIFFICULTIES

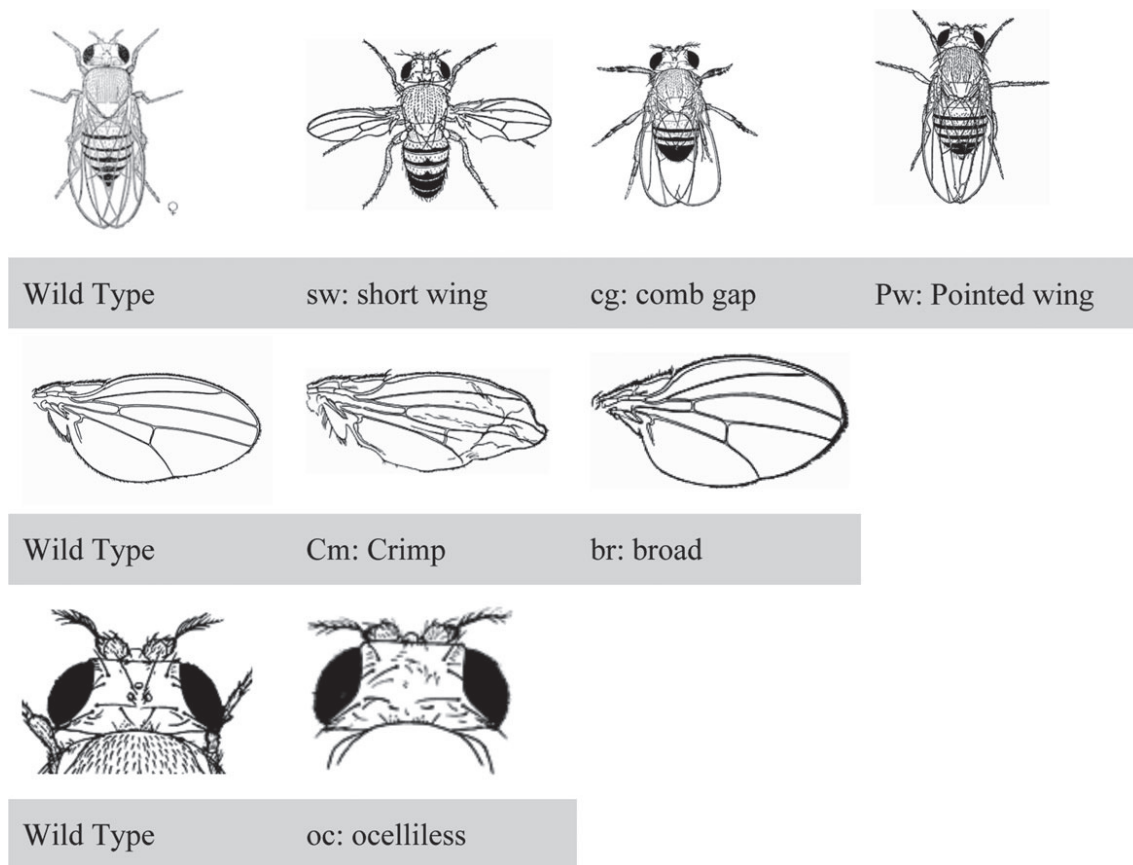
Only about 47% of the 1955 mutants examined were included in the final complexity dataset. The others were excluded from the dataset due to: (a) vague descriptions, making interpretation in complexity terms difficult or uncertain, (b) mutation irrelevant to adult gross morphology, such as behavioral or embryonic mutants, or (c) duplication of an already counted mutant phenotype, for example, there are around 670 alleles reported that affect Notch, resulting in many similar mutant descriptions of the same basic gross morphology. In a case like this, only the original Notch and variants with distinctive phenotypic difference were counted.

For the 916 usable mutants, terminology was not always consistent because of the variety of labs and researchers spanning time and space (see Lindsley and Zimm 1992 and [flybase.org](http://flybase.org) for a complete list of sources). To resolve this issue, any questionable morphological changes were investigated

**Table 3. Examples of *D. melanogaster* mutants and their complexity changes**

Mutation	Description	Complexity change	Focal level
sw: short wing <sup>1</sup>	Wings spread and incised with irregular veins	Part-type gain Shape gain	Part Part
eg: comb gap <sup>1</sup>	Shortening of some legs	Part-type gain Shape replacement	Part Part
Cm: Crimp <sup>1</sup>	Wings crimped irregularly	Part-type gain Shape gain	Part Part
Alp: Abnormal leg pattern	Fusion of metatarsal and second tarsal	Part-type replacement Part-type loss	Part Subpart
ty: tiny	Bristles small	Part-type replacement Shape replacement	Subpart Subpart
oc: ocelliless <sup>1</sup>	Ocelli completely absent	Part-type loss	Subpart
br: broad <sup>1</sup>	Wings broader and shorter with rounder tips	Part-type replacement Shape loss	Part Part
Pw: Pointed wing <sup>1</sup>	Wings narrowed slightly at tips	Part-type replacement	Part
U: Upturned	Wings upturned, dark, and wavy, eyes mottled with light flecks	Color gain (wings) Color gain (eyes) Shape gain (wings)	Part Part Part
wo: white ocelli	Ocelli colorless	Part-type replacement Color loss	Subpart Subpart
bre: bright eye	Eye color brighter red	Part-type replacement Color replacement	Part Part

<sup>1</sup>There is a corresponding illustration in Figure 3. Mutation descriptions are simplified for demonstration purposes.



**Fig. 3.** Six illustrations of *D. melanogaster* mutations, corresponding to Table 3, compared with the wild type (first column). Mutant illustrations all from Lindsley and Zimm (1992) except for the full adult wild-type illustration (top left), which is from Morgan (1919).

using <http://flybase.org> and then interpreted consistently throughout—e.g., comparing “upturned” and “upcurled” wings. A similar problem arose when determining the total number of added part types in certain mutations. For example, if legs are “irregularly shortened” or bristles are “abnormally deranged,” it is difficult to calculate the number of parts that have changed and the degree to which they have changed. How many of the six total legs are “irregularly shortened” and are those irregularly shortened legs different enough each to be considered distinct part types? This problem was minimized by interpreting the terminology in a consistent and conservative way. For example, if a description mentioned irregularly shortened legs, to be conservative, only half of the part type instances were assumed to change (i.e., only three legs—of the total six—were assumed to be irregularly shortened). The word “irregular” is interpreted as a unique change in each instance; thus, in this example, the three irregularly shortened legs would be counted as three distinct additional part types, producing a total of four leg part types (the original plus three unique legs of different length).

In cases that were too ambiguous to fit this rubric, we adopted another conservative interpretation, inferring fewer rather than greater increases in complexity. For example, with respect to

bristles, the above formula would result in a large number of new part types being added. If bristles were described as “abnormally deranged”, for instance, it was assumed that the original bristle part type was still present, as well as one or two new deranged-bristle part types, depending on the rest of the description. If the description was lengthy about the different types of bristle changes, this was interpreted as an additional two new part types; however, if “abnormally deranged” was the only description, then, for the sake of being conservative, it was assumed that those bristles that changed were “abnormally deranged” in a similar enough way to be considered the same part type.

With all of these difficulties, if errors were introduced by incorrect interpretations, they are expected to be random with respect to complexity; thus, there is no reason to think that any mistakes would produce a net bias toward either a loss or gain in complexity in the results.

### DEVELOPMENTAL BIAS

We need to address the possibility that positive results—greater complexity in mutants—might be biased by the developmental

component of selection. This would occur if mutant morphologies leading to part-type loss are more likely to be inviable. There is a standard line of argument in biology that says mutations are more likely to produce losses of part types than to produce gains (Saunders and Ho 1976), because mutations tend to disrupt, rather than create, the pathways that construct parts. Reinforcing this tendency is the hierarchical structure of development. Organisms start as simple embryos and become more complex, adding part types as they develop, with the result that early-occurring mutations that disrupt developmental pathways have the potential to eliminate whole suites of parts at a stroke. If such eliminations leave the embryo inviable, they would be absent from our dataset of *Drosophila* mutants, biasing our results toward gains. This argument has some merit; however, it is also widely acknowledged that (a) defects in development need not eliminate part types if pathways are well buffered, and (b) development is only imperfectly hierarchical. Defects in normal pathways can allow expression of underlying default pathways, leading to greater differentiation among parts. Further, the pathways leading to novel part types may be many, and more deviously ingenious, than we are accustomed to thinking. Even a loss of parts can have the secondary effect of producing new tissue contacts, and such contacts can produce new part types. For example, it has been shown that in bird evolution, the lateral spreading of a tendon from one bone surface to another produced an inductive event leading to a new part type, the syndesmosis tibiofibularis (Müller and Streicher 1989). Finally, a treatment of complexity by Oakley and Rivera (2008) offers a counterweight to the intuition that part-type gain must be difficult or less probable. They describe three standard routes by which part-type gain occurs: (a) copying and divergence (i.e., one part type giving rise to two); (b) splitting and divergence, where an ancestral part splits into two subparts which then diverge (again, one part type giving rise to two); and (c) copying or splitting followed by fusion, in which two ancestral part types either split or are copied and half of one pair fuses with half of the other, producing a third type. (See Oakley and Rivera 2008 for a number of examples in the evolutionary development of the vertebrate eye, spanning a hierarchical range from the single gene to the gene network to the tissue and organ level.) The point is that there are a number of simple developmental routes to new part types, all of them probably common in evolution.

At present, any answer to the question of whether or not there is any developmental bias against loss (or against gain, for that matter) must be speculative. Therefore, in the absence of compelling evidence one way or the other, we adopt the assumption that no bias exists, and conclude that the complexity of viable mutants is a representative sample. In other words, we assume that the structure of development does not bias our results in favor of the ZFEL.

## RESULTS

In examining the gross morphology of each *D. melanogaster* mutant compared to the wild type, every change—part-type, shape, and color—was categorized according to its effect on complexity—gain, loss, or no change. Among the 916 *D. melanogaster* mutants, there were 144 part-type gains and 24 part-type losses at the part level, and 119 part-type gains and 55 part-type losses at the subpart level (see Table 4). One mutant could gain or lose multiple part types, although it was typical for a part-type addition or subtraction to result in a gain or loss of only one part type (the mean part-type gain or loss of each mutant was  $1.0 \pm 0.3$ ). For shape, there were 485 gains and 39 losses at the part level, and 32 gains and only 3 losses at the subpart level. For color, there were 85 color gains and 6 losses at the part level, and 6 gains and 20 losses at the subpart level.

Table 5 presents the results of one-tailed *t*-tests of the null hypothesis that the frequency of gains is the same as that of losses against the alternative hypothesis that gains are more frequent. (A low *P* value in Table 5 represents a rejection of the null and supports the hypothesis that the frequency of gains is higher than the frequency of losses.) Part level part-type, shape, and color gains were significant ( $P < 0.0005$ ), and subpart level part-type and shape gains were significant ( $P < 0.0005$ ). Subpart level color losses were more frequent than gains; thus, if the null hypothesis (frequency of gains is the same as that of losses) is tested against the alternative hypothesis that losses are more frequent, subpart level color losses were significant ( $P = 0.0019$ ). This apparent anomaly in the data illustrates the independence of the part and subpart levels, and upon closer inspection, appears to be due, at least in part, to constraints on color changes. At the part level, the bias toward increases in color complexity was largely the result of changes in wing color,

**Table 4. Gains and losses in part-type, shape, and color complexity over two focal levels—part and subpart—in 916 *D. melanogaster* mutants from Lindsley and Zimm (1992)**

Focal level	Part-type gain	Part-type loss	Shape gain	Shape loss	Color gain	Color loss
Part	144	24	485	39	85	6
Subpart	119	55	32	3	6	20

**Table 5. Likelihood that the frequency of complexity gains is higher than the frequency of losses in *D. melanogaster* mutants**

Focal level	Part-type complexity		Shape complexity		Color complexity	
	Gain	<i>P</i> value	Gain	<i>P</i> value	Gain	<i>P</i> value
Part	13.19	<0.0005	37.08	<0.0005	16.59	0.0005
Subpart	5.20	<0.0005	8.63	<0.0005	−3.20	N/A

Likelihood assessed using one-tailed *t*-tests.

which can only increase in color complexity because wild-type *D. melanogaster* wings are completely transparent. The bias toward decreases in color complexity at the subpart level was largely due to tergites (abdominal segments) and ocelli (simple visual organs). (As we discussed in the last section, possibly there is a developmental constraint at work here, biasing mutations toward loss of pigment, although it is also possible that a combination of constraint and viability selection is involved, for example, that some correlated change associated with greater color differentiation is lethal at an early stage.) The fact that tergites lost pigmentation while the abdomen (in its entirety) did not, demonstrates that color complexity in abdomen parts changes independently.

Each *D. melanogaster* mutant was also categorized by degree of constraint. A mutant is strongly constrained if there is little to

no variation within the expression of a mutation (e.g., every rough mutant has eyes that are rough in the same way), and it is weakly constrained if the phenotypic expression of a given mutant is variable (e.g., in each forked mutant, bristles bend and split in different ways and to varying degrees). Of the 916 *D. melanogaster* mutants recorded for complexity changes, 447 were categorized as strongly constrained and 323 were categorized as weakly constrained (the rest were discarded due to ambiguity). Table 6 presents the number of complexity gains and losses over two focal levels in those mutants categorized as strongly constrained and those categorized as weakly constrained. Table 7 presents the results of one-tailed *t*-tests, similar to Table 5, except here mutants are split into two groups—strongly constrained and weakly constrained—in order to investigate whether the higher frequency of gains are specific

**Table 6. Gains and losses in part-type, shape, and color complexity over two focal levels—part and subpart—in 447 mutants categorized as strongly constrained (SC), and in 323 mutants categorized as weakly constrained (WC).**

	Focal level	Part-type gain	Part-type loss	Shape gain	Shape loss	Color gain	Color loss
SC	Part	13	12	135	26	18	2
	Subpart	35	26	9	1	3	6
WC	Part	114	12	264	8	44	0
	Subpart	54	18	16	11	2	2

**Table 7. Likelihood that the frequency of complexity gains is greater than the frequency of losses in those mutants categorized as strongly constrained (SC) and weakly constrained (WC)**

Focal level	Part-type complexity		Shape complexity		Color complexity		
	Gain	<i>P</i> value	Gain	<i>P</i> value	Gain	<i>P</i> value	
SC	Part	0.20	0.4231	11.64	<0.0005	5.81	<0.0005
	Subpart	1.16	0.1262	4.00	0.0016	−1.00	N/A
WC	Part	15.42	<0.0005	45.85	<0.0005	N/A	N/A
	Subpart	4.90	<0.0005	7.50	<0.0005	0.00	0.50

Likelihood assessed using one-tailed *t*-tests.



to one or both of these subgroups. Part-type gains were significant ( $P < 0.0005$ ) at the part and subpart levels in weakly constrained mutants only. Shape gains were significant ( $P < 0.0005$ ) at the part level in both weakly and strongly constrained mutants, and at the subpart level of weakly constrained mutants. Gains in color were significant ( $P < 0.0005$ ) only at the part level in strongly constrained mutants.

## DISCUSSION

The results of this study suggest the presence of a strong drive toward complexity in evolution. The evidence for this conclusion is that *D. melanogaster* laboratory mutants show a significant bias toward increasing complexity compared to the wild type (Table 5), and those categorized as weakly constrained show a significant bias toward increasing part-type complexity while strongly constrained mutants do not. The former tests the ZFEL hypothesis when selection is reduced, the latter when selection and constraints are both reduced.

Our findings also have implications for complexity in its broader, colloquial sense, what might be called adaptive complexity. Adaptively complex structures are those with many part types, or great differentiation among parts, which are also functional. It might seem at first glance that there is no interesting relationship between the two. Most of the pure complexity (e.g., the novel part types) in our dataset is the result of breakdowns of redundancy and failures of symmetry: for example, asymmetrical wings and irregularly shortened legs. And such breakdowns and failures may not sound like promising material out of which to build adaptive complexity. However, there are reasons to think they may be very promising. Indeed, for adaptive complexity they may be foundational. It may be just these sorts of variation that—on the rare occasion that they can be stabilized by selection—provide the raw material for the differentiation, for the “multipartedness,” that is fundamental to both adaptive and functional complexity.

For example, symmetry breaking was involved in the functional differentiation of claws in fiddler crabs, where the larger was adapted for specialized function in sexual competition (Palmer 2005). It was also undoubtedly critical in the evolution of left–right differences between the ears of certain owls, an asymmetry that enables owls to locate a sound source in the vertical plane by comparing the differing sound intensity and spectra between the two ears (Norberg 2002). Examples can be multiplied indefinitely, and given their multiplicity, it is hard to doubt the adaptive potential of symmetry failure. Even in *Drosophila*: is there any reason that a left–right asymmetry in the wings of a fly could not alter the flight pattern in ways that are locally advantageous in some ecological circumstance? (Perhaps one in which ordinary flight capability is unnecessary and erratic flight is a sexual releaser; there are many possibilities.) Likewise for the breakdown of redundancy (of which failure of symmetry

is just a special case). Who is to say that a mutant fly with one leg shorter than the others—suitably stabilized in development by selection—could not in some ecological context become the next adaptive innovation in *Drosophila*? Notice too that the logic of the ZFEL allows that variation can be initially neutral but does not require that it be neutral. Selection on a crab claw for one function combined with selection on the other claw for another function produces differentiation, and if these changes involve different selection pressures, then they are independent, i.e., they are random-with-respect-to-each-other, which makes the resulting increase in complexity an instance of the ZFEL.

The ZFEL and selection may combine in a very different way to produce adaptive complexity. Recent work by Finnigan et al. (2012) revealed a case in which initially neutral, ZFEL-driven differentiation in a duplicated gene became irreversibly locked in by selection. The case involves a fungal ATPase, which ancestrally consisted of a six-member ring with five subunits of one type and one subunit of a second type, in other words, two part types. In fungi, drift in a paralogous gene for one of the five subunits produced a new subunit type—a third part type—with binding properties permitting it to insert between the first and second types, allowing perfectly functional rings to form. However, with the addition of this third subunit type, drift in the first subunit type destroyed its now-unused ability to bind to one side of the second type. Apparently, selection then opposed loss of the now-essential third part type, locking it in. This general process has been given the name “constructive neutral evolution” (Force et al. 1999; Stoltzfus 1999; Gray et al. 2010), which is somewhat misleading in that only the ZFEL-driven differentiation step is neutral. Selection (against removal of the third part) is essential to the lock-in process. In any case, this principle may turn out to be quite significant in the evolutionary growth of molecular complexity (Doolittle 2012). Further, there is no reason that the same principle could not operate at the level of gross phenotype, among cells, tissues, organs, and so on, as well as at the molecular level, with ZFEL-driven neutral variation among part copies leading to selection-driven lock-in of novel part types. From this perspective, any one of the various novel part types in *Drosophila* mutants that is sufficiently neutral—however unpromising it may seem—appears as at least a candidate for future lock-in.

In sum, what our results here for *Drosophila* do, and more generally what the ZFEL does, is offer a new perspective on the evolution of adaptive complexity, a perspective that: (a) draws attention to the enormous redundancy in organisms; (b) highlights the fact that the breakdown of redundancy is the expectation; (c) reveals the breakdown of redundancy to be a generator of complexity; and (d) raises the possibility that this complexity may be the stuff out of which adaptive complexity is constructed by natural selection.

This study also shows one route to broader empirical testing of the ZFEL hypothesis. The same methodology could be used to investigate the large dataset available on mutant phenotypes in

*Zea mays*. A similar method could be used to investigate morphological changes occurring under reduced selection in the eyes of cave organisms. Future testing of the ZFEL hypothesis in other taxa will help establish a domain of application, and move us closer to evaluating the ZFEL hypothesis more conclusively.

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