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The relationship between development and evolution through heritable variation

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Abstract. Darwin's theory of evolution by natural selection states that evolution occurs through the natural selection of heritable variation. Development plays the key physiological role connecting the heritable genotypes, passed from one generation to the next, to the phenotypes that are made available for selection. While at times the developmental variations underlying a selected trait may be neutral with respect to selection, it is through its effects on heritable variation that developmental tinkering affects evolution. We can gain a deeper understanding of the evolutionary process by considering the role of development in structuring variation and, through its effects on variation, structuring evolution. Both evolutionary theory and empirical studies show that features that interact in development tend to be inherited together and, hence, to evolve together. Gene mapping studies show that this modular inheritance pattern is due to modular pleiotropic gene effects, individual genes affecting a single modular unit, and that there is heritable variation in the range of features encompassed by these modules. We hypothesize that modular pleiotropic patterns are sculpted by natural selection so that functionally-and developmentally-related traits are affected by module-specific genes.

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The developmental process provides the physiological relationship between genotype and phenotype encompassing the means by which genetic and environmental factors affect the developing phenotype. However, evolution is not directly affected by this physiological relationship. Instead, evolution depends on the statistical relationship of genotype to phenotype. Even so, the physiological relationship between genotype and phenotype structures this statistical relationship. To relate development to evolution, we need to relate the physiological bases of development to the statistical relationships between genotype and phenotype.

Over a decade ago, Atchley & Hall (1991) proposed a model relating developmental processes to the statistical relationship between genotype and phenotype

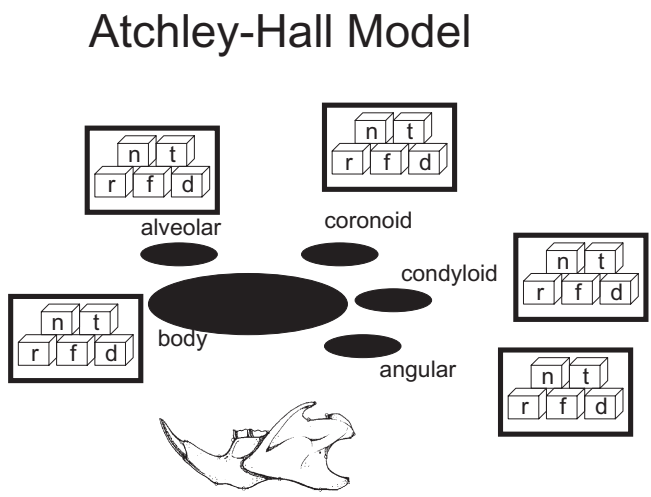

FIG. 1. The Atchley-Hall Model for the developmental basis of variation in morphological size and shape. The mandible is composed of five cellular condensations (coronoid process, angular process, condyloid process, body, alveolus). The final size of each condensation depends on five developmental parameters: n (number of cells in condensation), t (time of condensation initiation), r (rate of cell division), f (fraction of cells mitotically active), d (rate of cell death).

(see Fig. 1). In this model, the size of a developing organ at some time '*t*' (n_t) was considered as a function of the number of cells (n_0) and the time (t_0) of the initial condensation, the rate of division of individual cells (r), the fraction of cells mitotically active (f), and the rate of programmed cell death (d),

$$n_t = n_0(1 + (t - t_0)rf - (t - t_0)d).$$

The genetic variance of an organ's size and shape would then be a function of the genetic variances and covariances among these developmental parameters, thereby relating variation in the developmental parameters to the variation in the phenotype.

Developmental homoplasy

Using this model, Atchley et al (1997) noted that any given phenotypic value (n_t) could be obtained through a variety of combinations of the developmental parameters. Hence, specific developmental parameters may be neutral in relation to selection on the final phenotype. They referred to this phenomenon as developmental homoplasy, many different developmental mechanisms leading to the same phenotypic end result. In this situation, the developmental underpinnings of the evolved trait depend solely on the genetic variances of the developmental

parameters and their genetic covariances with the selected trait, the end phenotype. While it is certainly possible that the developmental parameters themselves could be subject to selection, in the situation where selection is on the end phenotype, the precise developmental mechanisms underlying evolutionary change are neutral, and hence not specifically important for an understanding of the evolutionary process.

Two examples of developmental homoplasy serve to illustrate the general principle. Oxnard (1976, 1984) studied morphological variation in relation to locomotion in prosimian primates. He contrasted species from genera exhibiting generalized quadrapedal locomotion (e.g. *Lemur*) with vertical clinging and leaping species (e.g. *Indri*, *Avahi*, *Propithecus*, *Galago*, *Tarsius*). The leaping species have relatively long legs which are commonly interpreted as an adaptation to leaping forms of locomotion because the force exerted during leaping is proportional to the change in leg length during lift off. Longer legs lead to more powerful leaps. However, a multivariate analysis of limb segments in these forms indicated that while the leaping prosimians all had relatively long legs, they accomplished this through different developmental mechanisms. Members of the genera *Indri*, *Avahi* and *Propithecus* have relatively long femora and metatarsus, the first and last elements in the limb, while *Galago* and *Tarsius* have relatively long middle segments, tibia and tarsus. It is important to note that both these groups represent multiple, independent origins of long legs and leaping. Selection for increased leg length in leaping prosimians resulted in the evolution of different limb elements in different groups. Which elements increased in length in these groups was likely determined by which developmental variations happened to be present in their ancestral forms rather than representing a specific adaptation.

A second example of developmental homoplasy is the result of a selection experiment for longer tails in mice. Rutledge et al (1974) formed two small initial populations from a large random-bred mouse population to serve as replicate selection lines for increased tail length. The experiment succeeded and both selected lines evolved similarly long tails. However, upon further examination they found that one line accomplished this by making individual vertebra longer while the other increased the number of vertebra in the tail. This diversity in developmental response was due to random founder effects involved in the formation of the original selected lines. One line happened to vary for vertebral length and the other for vertebral number. Different developmental processes underlay the common response to selection for longer tail length.

Elements of evolutionary theory

Even though in these examples the mechanisms underlying development are neutral, they can still have consequences for further evolution. Development may

have a prospective effect on future evolution even when it is neutral in retrospect. How does development intersect with evolutionary processes? To answer this question, we need to consider basic evolutionary theory at the level of first principles.

Darwin's theory of evolution by natural selection states that evolution occurs through the natural selection of heritable variation. Natural selection is due to a correlation between fitness and some phenotype caused by the interaction between phenotype and environment. Heritable variation refers to the heritable differences between individuals. In the early 20th century evolutionary geneticists put Darwin's theory on a clear, logical and mathematical basis (Provine 1976). They showed that the heritable variation in a population was measured by the additive genetic variance. When many traits are considered together, heritable variance takes the form of a square symmetric matrix with additive genetic variances along the diagonal and additive genetic covariances between traits off the diagonal, the genetic variance/covariance matrix (G). The additive genetic variance measures the amount of heritable variance for the trait while the additive genetic covariances measure the degree to which different traits are inherited together. This co-inheritance is due to pleiotropy, where one gene affects several traits, and/or linkage disequilibrium, where two different genes, each affecting a separate trait, tend to be inherited together most often because they lie near each other along the chromosomes. In seeking the interface between development and evolution perhaps the most fruitful enquiry considers the effects of developmental processes in moulding patterns of heritable variation.

Heritable variation is itself an evolving property of a population. Two major factors affect the evolution of heritable variation; the pattern of new variation produced by mutation and the pattern of stabilizing selection on the traits (Lande 1980). While mutation may be random with respect to selection, it is unlikely that all traits mutate at the same rate or mutate independently of each other. Mutation will produce high variance in some traits and lower variance in others. Likewise, a range of traits will be affected by the pleiotropic effects of new mutations while other traits are unaffected. Theoretical predictions for the evolution of the mutation variance/covariance matrix are not available but it seems possible that the effects of mutations are channelled by developmental processes. Stabilizing selection also causes the evolution of heritable variance/covariance patterns. Traits that interact during development or to perform an adult physiological function will be co-selected because the fitness effect of one trait depends on the value of the second trait. This is sometimes referred to as internal stabilizing selection because the selection is on the relationships of the parts rather than on their direct interface with the environment (Reidl 1976). The selection is to 'fit in' with other aspects of the phenotype. For example, the upper and lower jaws work together in mammalian mastication for food preparation. In a certain instance, it may not matter

whether the mandible itself is long or short so long as the upper and lower jaws match, preventing over- and underbite. Selection would act strongly against mismatches, long mandible with short maxilla and vice versa, selecting to correlate the inheritance of these two traits.

Morphological integration

The logic of these relationships is commonly considered under the principle of morphological integration (Olson & Miller 1958). Morphological integration refers to the relationships between morphological parts. Olson & Miller (1958) postulated that developmentally and/or functionally related traits would be correlated in their distributions within populations and evolve jointly rather than mosaically. Later quantitative genetic theory supported their arguments by indicating that functionally and developmentally related traits should be inherited together because patterns of stabilizing selection lead to the co-inheritance of related traits (Lande 1980, Cheverud 1982, 1984) and that because of this co-inheritance, functionally and developmentally related traits should evolve together through correlated responses to selection (Lande 1979). The phenotype is integrated, not atomistic.

In a series of studies on the co-inheritance of cranial morphology in non-human primates (Cheverud 1982, 1996a), it was indeed found that functionally and developmentally-related cranial traits are relatively highly genetically correlated. Related traits share about twice the variance shared by unrelated traits. This general pattern is also evident in the phenotypic patterns of cranial integration throughout the primates (Marroig & Cheverud 2001, Ackermann & Cheverud 2004). The primary source of this patterning is a contrast between the braincase and face of the skull. The primate braincase grows early in life, in step with the growing brain that it serves to enclose and protect. In contrast, the face, and especially the masticatory apparatus, grows later, along with the rest of the body. Both genetic and phenotypic correlations are higher within these parts of the skull than between them. They are individuated relative to one another forming separate cranial modules.

Modular or antagonistic pleiotropy

Co-inheritance of developmentally and functionally related traits can occur through two different genetic mechanisms; pleiotropy can have an antagonistic pattern with each locus affecting each trait but with opposite effects on different developmental regions or processes, or it can display a modular pattern, with the effects of subsets of genes being restricted to specific subsets of developmental units (see Fig. 2). These two possibilities reflect different styles of developmental processes

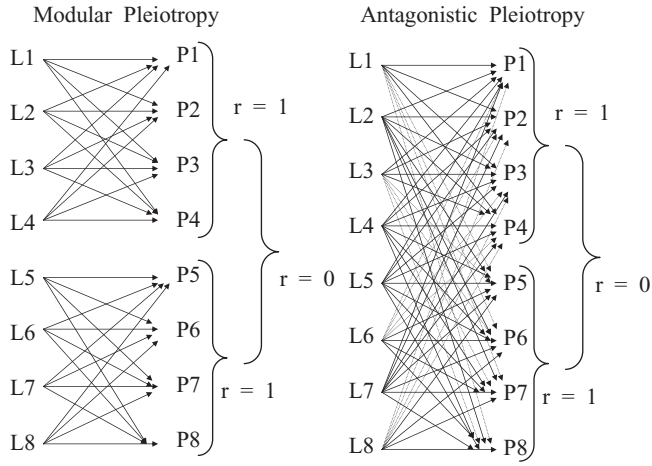

FIG. 2. The two possible pleiotropy patterns responsible for genetic morphological integration; modular pleiotropy and antagonistic pleiotropy. L*i* refers to different gene loci and P*i* refers to different phenotypic traits. In antagonistic pleiotropy each locus affects all traits but has opposite effects on different modules. In modular pleiotropy the effects of loci are restricted to a subset of traits.

(Riska 1986) and have divergent evolutionary consequences. However, both can account for the observed co-inheritance of functionally and developmentally related traits.

In order to distinguish between these two possibilities, it is necessary to examine the effects of individual gene loci on morphological characters. This has become possible over the past decades through quantitative trait locus (QTL) analysis (Lynch & Walsh 1998). In a typical experiment of this kind, two inbred strains are crossed to produce F_1 hybrid animals that are heterozygous at all loci different between the two parent strains. The F_1 animals are then intercrossed to produce a F_2 generation. In the F_2 generation genes will segregate according to Mendel's Laws as will any phenotypes they affect. The effects of specific gene regions on morphology can then be discerned by correlating the segregation of genes with the segregation of phenotypes across the whole genome.

We have performed several QTL experiments where we have measured the pleiotropic effects of genes specifically to determine whether modular or antagonistic pleiotropy plays a major role in structuring heritable variation. In a study of cranial morphology, Leamy et al (1999) found that most genes had modular effects with respect to the face and braincase. They found eight QTLs affecting only the face, eight QTLs affecting only the braincase, eight QTLs affecting the entire skull, and two QTLs with antagonistic pleiotropic effects between the face and

braincase. Further, more anatomically detailed QTL studies are underway both on mouse and baboon crania.

In other studies of the developmentally complex mandible (Atchley & Hall 1991), we again found a modular pattern with most loci having their effects restricted either to the ascending mandibular ramus with its muscle attachment regions or to the tooth-bearing alveolus (Cheverud et al 1997, Ehrich et al 2003). These results strongly support the hypothesis of modular pleiotropy. Kenney-Hunt and colleagues (personal communication) are currently testing the modularity hypothesis across the whole skeleton in a hierarchical fashion, contrasting the appendicular and axial skeleton, and their component anatomical subsets.

Genetic variation in pleiotropy and differential epistasis

The question then arises as to how modular pleiotropy has evolved. If it has evolved through natural selection or genetic drift, the pleiotropic range of gene effects must itself be genetically variable. The pleiotropic effects of a gene can vary because of differential epistasis (Cheverud 1996b, Cheverud et al 2004). Epistasis occurs when the phenotypic effects of a gene vary depending on the genotypes present at another locus. If these epistatic effects differ from one trait to another, there is differential epistasis and genetic variation in the range and strength of pleiotropic effects (see Fig. 3).

The phenomenon of differential epistasis is well known in the genetics literature as the following examples illustrate. The abnormal abdomen mutation (aa) in *Drosophila* was long known in the laboratory and named for its effect on abdominal cuticle development, resulting in a juvenilized cuticle (Templeton et al 1985, 1993, Templeton & Johnston 1988, Hollocher et al 1992). In addition, 'aa' had pleiotropic

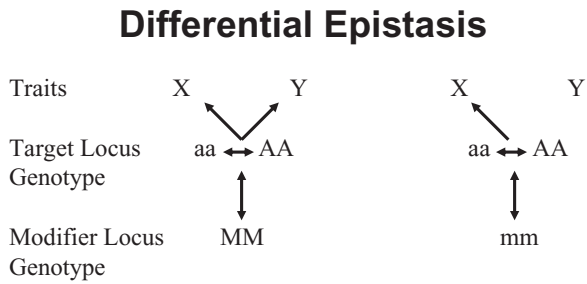

FIG. 3. Differential epistasis occurs when the pleiotropic range of traits affected by a locus varies depending on genotypes at a second, modifier locus. In this example the difference between homozygotes at the A locus affects both traits X and Y when the modifying locus has the MM genotype but the A locus effects are restricted to trait X when the modifying locus has the mm genotype. The mm genotype suppresses the effect of the A locus on trait Y.

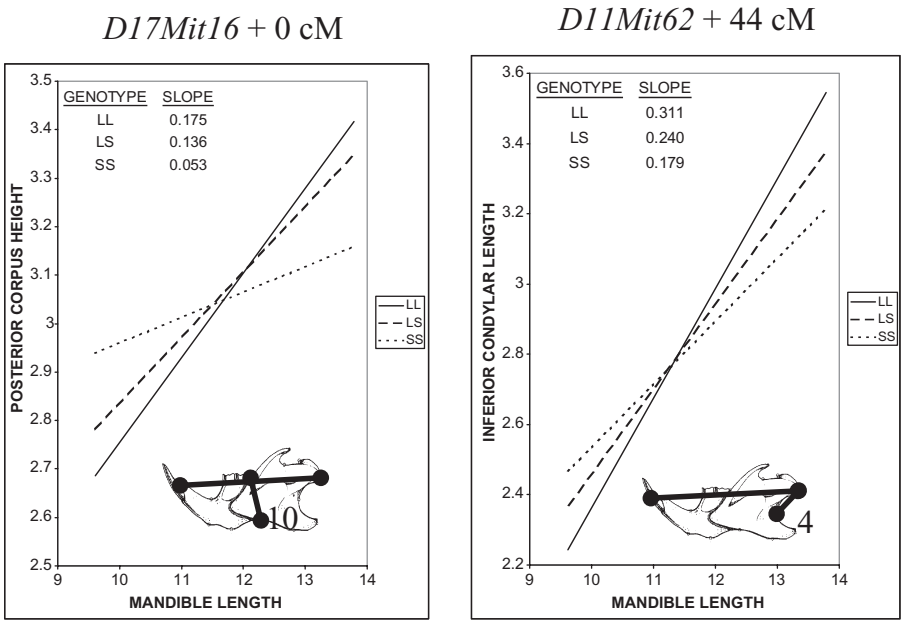

FIG. 4. Relationship quantitative trait loci (QTL) on chromosomes 17 and 11 affecting the relationship between mandible length and posterior corpus height and inferior condylar length, respectively. In each case, the slope of the regression of the specific mandibular region on total mandibular length changes depending on the genotype at the locus identified. These QTL are a source of genetic variation in pleiotropy. In each case the LL homozygote leads to a relatively small local mandibular region in short mandibles but leads to a relatively large region if the mandible is long.

effects on life history traits, including slow development time, higher early fecundity, and low adult survivorship. The same molecular mutation was discovered in a population in Hawaii living across a severe moisture gradient. However, its pleiotropic effects had been modified by epistatic interactions with other loci. The effect on the cuticle had been suppressed while the life history effects were maintained. The modified 'aa' mutation was selected for in dry conditions due to its adaptive life history effects. However, selection also acted to modify pleiotropy at the locus due to epistatic interactions by evolving a genetic background in which the deleterious juvenilized cuticle was suppressed because it is maladaptive in dry environments.

A second example comes from the agricultural research community (Geetha et al 1991, Moro et al 1996, Burnett & Larkins 1999). The opaque-2 maize mutant greatly enhances the amount of lysine in the endosperm. Lysine is an essential amino acid for humans and could enhance the nutritional value of corn. Unfortunately, the mutation also resulted in a thin seed coat that interfered with modern

harvesting techniques. Researchers used backcrossing with recurrent selection to produce a thicker seed coat while maintaining the high lysine content effect of opaque-2. Through this selection, alleles at several loci that epistatically suppressed opaque-2's effect on the seed coat while maintaining its effect on lysine content increased in frequency. Again, the pleiotropic effects of the opaque-2 gene were modified by evolution at epistatically interacting loci.

Relationship quantitative trait loci

The phenomenon of differential epistasis can be seen in the effects of relationship QTLs (Fig. 4). Relationship QTLs are regions of the genome that affect the relationships between traits with alternate alleles producing stronger or weaker ties between phenotypes. Relationship QTLs provide genetic variation in the range of pleiotropy expressed at a locus (see Figs 2 and 3). We mapped relationship QTLs across the mouse genome affecting the relationship between individual parts of the mandible and overall mandibular size (mandible length; Cheverud et al 2004). We uncovered a total of 23 QTLs across 13 different chromosomes. About one-third of these QTLs also had direct effects on the mandible (Ehrich et al 2003). The principal of morphological integration was also expressed in the patterns of traits affected by pleiotropic relationship QTLs. At most genomic sites, the traits with a genetically variable relationship with mandible size consisted of sets of developmentally and functionally related traits. These loci produce genetic variation in the individuation of mandibular regions with respect to the mandible as a whole, or genetic variation in modularity. Such variations are critical for models of the evolution of developmental modules and the individuation of parts in evolution.

These results lead us to the hypothesis that the observed modular pleiotropic patterns are sculpted by natural selection so that functionally and developmentally related features are affected by module-specific genes, freeing the population phenotypic mean to evolve by masking deleterious pleiotropic effects.

Summary

I suggest that the most important relationship between development and evolution occurs through the effects of developmental processes and interactions on patterns of heritable variation as measured by the genetic variance/covariance matrix. We can predict patterns of heritable variation based on developmental relationships among traits, as realized through patterns of mutational variation and internal stabilizing selection. We found that developmentally related traits tend to be inherited together in a modular fashion because of modular pleiotropy and that genetic variation in the range of pleiotropic effects exists allowing for the evolution of module-specific gene effects.

Acknowledgements

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References

Ackermann RR, Cheverud JM 2004 Morphological integration in primate evolution. In: Pigliucci M, Preston K (eds) Phenotypic integration: studying the ecology and evolution of complex phenotypes. Oxford University Press, Oxford, p 302–319

Atchley WR, Hall BK 1991 A model for development and evolution of complex morphological structures. Biol Rev 66:101–157

Atchley WR, Xu S, Cowley DE 1997 Altering developmental trajectories in mice by restricted index selection. Genetics 146:629–640

Burnett RJ, Larkins BA 1999 Opaque-2 modifiers alter transcription of the 27 kDa g-zein genes in maize. Mol Gen Genet 261:908–916

Cheverud JM 1982 Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516

Cheverud JM 1984 Quantitative genetics and developmental constraints on evolution by selection. J Theor Biol 110:155–172

Cheverud JM 1996a Quantitative genetic analysis of cranial morphology in the cotton-top (*Saguinus oedipus*) and saddle-back (*S. fuscicollis*) tamarins. J Evol Biol 9:5–42

Cheverud JM 1996b Developmental integration and the evolution of pleiotropy. Am Zool 36:44–50

Cheverud JM, Routman EJ, Irschick DK 1997 Pleiotropic effects of individual gene loci on mandibular morphology. Evolution 51:2004–2014

Cheverud JM, Ehrich TH, Vaughn TT, Koreishi SF, Linsey RB, Pletscher LS 2004 Pleiotropic effects on mandibular morphology II. Differential epistasis and genetic variation in morphological integration. J Exp Zool B Mol Dev Evol 302B: 424–435

Ehrich TH, Vaughn TT, Koreishi SF, Linsey RB, Pletscher LS, Cheverud JM 2003 Pleiotropic effects on mandibular morphology I. Developmental morphological integration and differential dominance. J Exp Zool B Mol Dev Evol 296B: 58–79

Geetha KB, Lending CR, Lopes MA, Wallace JC, Larkins BA 1991 Opaque–2 modifiers increase g-zein synthesis and alter its spatial distribution in maize endosperm. Plant Cell 3:1207–1219

Hollocher H, Templeton AR, DeSalle R, Johnston JS 1992 The molecular through ecological genetics of abnormal abdomen IV. Components of genetic variation in a natural population of Drosophila mercatorum. Genetics 130:355–366

Lande R 1979 Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33:402–416

Lande R 1980 Genetic variation and phenotypic evolution during allopatric speciation. Am Nat 116:463–479

Leamy L, Routman EJ, Cheverud JM 1999 Quantitative trait loci for early and late developing skull characters in mice: A test of the genetic independence model of morphological integration. Am Nat 153: 201–214

Lynch M, Walsh B 1998 Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, Massachusetts

Marroig G, Cheverud JM 2001 A comparison of phenotypic variation and covariation patterns and the role of phylogeny, ecology and ontogeny during cranial evolution of New World Monkeys. Evolution 55:2576–2600

Moro GL, Habben JE, Hamaker BR, Larkins BA 1996 Characterization of the variability in lysine content for normal and opaque2 maize endosperm. Crop Sci 36:1651–1659
Olson EC, Miller RL 1958 Morphological Integration. University of Chicago Press, Chicago
Oxnard CE 1976 Primate quadrupedalism: Some subtle structural correlates. Yearb Phys Anthropol 20:538–553
Oxnard CE 1984 Order of man: A biomathematical anatomy of primates. Yale University Press, New Haven, Connecticut
Provine WB 1976 The origins of theoretical population genetics. University of Chicago Press, Chicago
Reidl R 1976 Order in living organisms. John Wiley & Sons, New York
Riska B 1986 Some models for development, growth, and morphometric correlation. Evolution 40:1303–1311
Rutledge JJ, Eisen EJ, Legates JE 1974 Correlated response in skeletal traits and replicate variation in selected lines of mice. Theor Appl Genet 45:26–31
Templeton AR, Crease TJ, Shah F 1985 The molecular through ecological genetics of abnormal abdomen in Drosophila mercatorum. I. Basic genetics. Genetics 111:805–818
Templeton AR, Johnston JS 1988 The measured genotype approach to ecological genetics. In: de Jong G (ed) Population genetics and evolution. Berlin:Springer-Verlag p 138–146
Templeton AR, Hollocher H, Johnston JS 1993 The molecular through ecological genetics of abnormal abdomen in Drosophila mercatorum. V. Female phenotypic expression on natural genetic backgrounds and in natural environments. Genetics 134:475–485

DISCUSSION

Hall: One of the complications of doing this on the mandible is that you had to do it on every cell population. Ideally, for the long bones, you would want to do it on each long bone of the limb. I assume you are going to start with just one, though?

Cheverud: We'll start with what looks promising from the pattern of effects. Some of those QTLs affected all of the long bones, and presumably all of their growth plates could be affected. We might sample some of their growth plates for that purpose. Others are specific to an individual bone or pair of bones. There is a nice femur/humerus QTL. This would restrict our examination of the fetus by the adult morphology we see. Sometimes the preparation is difficult: it can be hard to get both ends of the bone prepared properly. We use genetically replicable animals, and if the lower and upper growth plate are from different animals we will still be able to compare them.

Hall: That's nice to have that genetic homogeneity.

Wagner: If you hypothesize that the modular pattern of pleiotropy is evolving, then from what we understand from models that involve epistasis what is needed to evolve such genetic architecture is directional epistasis. Specifically, one would expect that between functional groups there is more negative epistasis than within the same group. Do you find any differences in the kinds of epistatic interactions among different groups of traits?

Cheverud: There is a lot of variety in the style of epistasis, and exactly which genotypes are involved in the interaction. This is a subject for future research. A lot of it seems to be that the epistasis will be present or absent for different traits. There will either be some or none. Some of this variability might have to do with suppressing epistasis on one of the traits, so it no longer shows the epistatic effect. Indeed this should result in the presence of more suppressing epistasis between functional groups.

Hallgrimsson: When rates change in the growth plate, do you expect the same developmental processes to be involved as might occur in a simple system, or can growth plates evolve different rates of growth by different developmental means? Can growth rate in the growth plate evolve by different developmental processes?

Cheverud: Yes, the growth plate can evolve by different developmental processes. Getting at this relies on making congenic lines where you isolate that one part of the genome from all of the rest. Then you could study in detail the basis for the growth rate variation at a particular QTL.

Hallgrimsson: You pointed out the increased size of the proliferative zone as one way of getting a faster rate of growth. When you do a cartilage-specific knockout of PTEN, which inhibits mitosis, you get the opposite: a faster growth rate, a shorter proliferative zone and a bigger hypertropic zone.

Cheverud: There are many different ways to get to the same place, again. It will be interesting to see whether some are used more than others.

Duboule: Over the past 20 years in developmental biology we have learned that in the vertebrae, which is a perfect example of a modular structure with several ossification centres, the mechanism that regulates the number of cells that condensate for a given ossification centre, and the mitotic index of these cells, all these mechanisms are either the same or tightly linked. Personally, I don't see how you can reconcile this with the fact that you can get to the same point with different mechanisms. I have the same question with regard to the tail. Getting a long tail by itself cannot provide any adaptive advantages. What may provide an advantage is what you do with your tail. You will be able to do different things if you have long vertebrae within the tail or a collection of short vertebrae. I don't think the question is whether you can get two long tails with different mechanisms; the question is whether these tails will achieve the same kind of performance. My answer would be no.

Cheverud: Relative to the selection criterion, tail length, the two populations did achieve the same performance. One of the major factors of tail length is giving off heat. It is hard to see how the size or number of vertebrae would affect that. If they were hanging from it or using it to generate force, I could see a tremendous possibility for difference. In relation to your first comment, if these different sources of developmental variability are indeed tightly correlated due to pleiotropy,

our QTLs should affect several developmental processes rather than just one. This will be an interesting outcome from the gene mapping.

Stern: You are making strong inferences about the evolvability of these systems from experiments that are very unlike those that would occur in natural populations. You are starting with small populations and applying strong selection pressure on one aspect of the phenotype at a time. You are pulling out variants that may be able to construct superficially similar phenotypes in different ways. It is not at all clear that in natural populations selection is going to be trying to isolate one phenotype from the rest of the organism.

Hanken: A natural analogue of the phenomenon that Rutledge et al (1974) produced in the lab is seen in two genera of neotropical salamanders that comprise extremely elongated animals selected for living underground (Wake 1966). All have tiny reduced limbs and elongated trunks and tails. One genus (*Lineatriton*) has accomplished this by retaining the ancestral number of vertebrae (as seen in non-elongate genera), but each vertebra is elongated. The other genus (*Oedipina*) has done this by increasing the number of vertebrae. In this case the selection seems not to be for specialized tail function as much as for just an elongated body.

Cheverud: The difference in response could have functional consequences for future interactions with the environment. There would be a prospective effect of a developmental process that happens to have evolved.

Hanken: In Rutledge's experiment, selection for increased tail length yielded either more vertebrae or longer vertebrae; results could have gone either way. In the case of neotropical salamanders, there are many different genera yet most retain the same ancestral number of vertebrae. This appears to be a constrained feature, and *Oedipina*, the one genus that has increased the number of vertebrae, seems to have escaped the constraint. There is a biased distribution of the phenotype.

Cheverud: It would be interesting to see how this genus broke free of that constraint developmentally.

Brakefield: I have a thought experiment. In the mouse example, if you were to select using much larger sample sizes, would you expect to see a mixed strategy evolving? Posing this question a different way: Why don't they evolve longer tail length by doing it both ways together?

Cheverud: As you say, they could well evolve using both processes in a larger population with a smaller founder effect. Testing that though would be difficult because the real answer to that question is 67 cents a cage per day! Experiments are limited in size by practical considerations.

Wagner: Since these two traits are additive, the number of vertebrae and their size, as you start selecting and one of them is responding, the variance of this character is also increasing because of the scaling relationship between the variance and mean. This leads to symmetry breaking, so that the selection response of the character that responded first will continue to have a higher rate of response.

If you have multiple variables that add to the same phenotype and start selecting on one of them, it becomes a more effective target of selection. I suspect the fact that even in nature we don't see intermediates has to do with some kind of symmetry breaking.

Cheverud: This would occur with direct selection on the developmental processes themselves, not on the end phenotype.

Hall: That is interesting, because there is a distinct difference in timing between producing extra vertebrae (very early, when the somites are segmented) versus increasing length (much later in the life cycle).

Weiss: There are other precedents, such as Mackay's work on bristle number, Richard Lenski's work with bacteria and selection for malaria resistance in humans. They all show some shared mechanisms, but also different mechanisms. This supports the idea that evolution will pick whatever is there.

Oxnard: I want to return to the modularity issue. That work was done in rodents, which only have incisors and molars. Two neural crest cell populations were found relating to the two alveolar units containing those teeth. I have often wondered if two populations of cells were seen (i.e. two units) because intervening units were lost because of dental reduction. Our morphometric studies in primates (which have incisors, canines, premolars and molars) implied that there were four such alveolar units, one for each group of teeth. This seems to support the 'populations of cells idea', i.e. units or modules. However, even in living primates there are 'missing' teeth. There are many incisors missing (as many as six) if we are to believe the fossil record. Again, though living primates have two premolars; there are many more in some fossil primates. There are even many more molars in some fossil primates. If we had a primitive primate that had all of these, would we still see separate populations of crest cells and separate units of the alveolar process, or would we see something that really was just a spectrum? Would the situation it still be modular or would it be something more continuous, linear and quantitative? Is the modularity artificially produced by dropout of teeth? Could it have been linear originally?

Wagner: That is logically not consistent. If you consistently drop out say the third tooth, and then the eighth and sixth, then there has to be a modular system. If it was quantitative, you would just reduce them and still have a gradient of morphology.

Oxnard: Were there still modules in the bones when there was a full suite of teeth? There might well have been, particularly because the other parts of the mandible seem to have been modular. However, loss of teeth may impose an apparent modularity upon the bony processes which may actually have been continuous. Though our morphometric studies imply that each tooth is a unit, the bone in which they sit (and which is produced by totally different developmental processes) is more of a 'rubber' structure—that is, it can change shape in a continuous fashion.

Cheverud: It would have been modular if you could identify them as being these differentiated types of teeth.

Lieberman: How many strains of mice were the selection experiments done on?

Cheverud: For the tail experiment there were two up, two down.

Lieberman: It might have been interesting to do the experiments on different strains to see how the different backgrounds correlated.

Cheverud: You would certainly get different kinds of results as the QTL replication between different strain pairs is low.

Hall: It would be useful to look at the range and type of variation between the strains.

Bell: One of the things people are interested in is how labile pleiotropies are compared to individual traits. If pleiotropies are really stable features of lineages, this could constrain evolution over long periods. If they are labile, it doesn't really matter: they are not constraints.

Cheverud: The answer will be somewhere in the middle.

Lieberman: If you look at domesticated animals, there must be some conserved pleiotropies out there.

Cheverud: At least we found variation in them. If variation is there it can be selected on.

Budd: You touched on some general questions which have been much discussed in the Evo-Devo literature. These are topics like the relationship between modularity in the phenotype and genotype: which one comes first and how do they both evolve? You seem to be suggesting a way out of this problem with your hypothesis of how natural selection works to produce these pleiotropic effects and modularities. Do you see this as a general answer?

Cheverud: In general there is no answer to the question of whether the genotype or phenotype does it first: they both do it together at the same time, as part of the same system. The separation of the two into different parts, with one operating independently of the other, is wrong.

Weiss: If we look at modern human variation and the large number of studies that have been done on disease, they have shown that mapping results are hard to replicate. Usually a few chromosomal locations are replicated, but they end up explaining a small fraction of the variation. Another characteristic is that the more precisely defined the phenotype the fewer locations, but they are still mostly not replicable. The more sharply you define the trait the closer you get to something genetic. It makes a lot of sense: if you are getting close to something tied to a gene product, you are going to map to the same region again and again; if you are looking more broadly you aren't.

Cheverud: When formal studies have been done of the overlap of different strain crosses, replicated results haven't been obtained. Background effects seem to have

a big role in which genes are displayed for natural selection or for phenotypic variation.

Lieberman: So often people interpret this as noise.

Oxnard: I was delighted to see the reference to our work on leaping. But the devil is in the detail. In primates, there are three different forms of leaping; different mechanical modes in different ecological situations associated with quite different anatomical adaptations. In each of these leaping types the species are from at least two widely separated phylogenetic groups. This immediately implies that there is an enormous amount of parallelism or convergence in the story.

References

Rutledge JJ, Eisen EJ, Legates JE 1974 Correlated response in skeletal traits and replicate variation in selected lines of mice. Theor Appl Genet 45:26–31

Wake DB 1966 Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem South Calif Acad Sci 4:1–111