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Developmental Integration and the Evolution of Pleiotropy[1]

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SYNOPSIS. The different forms of morphological integration, developmental, functional, genetic, and evolutionary are defined and their theoretical relationships explored. Quantitative genetic models predict that the co-selection of traits involved in a common function will lead to pleiotropic effects at the loci affecting them while functionally-unrelated traits will be affected by separate sets of loci (Wagner, 1996). The patterns of genetic variation produced by these pleiotropic mutations and stabilizing selection for functionally and developmentally interacting traits results in their specific co-inheritance relative to other traits. This in turn leads to their co-ordinated response to selection. Therefore, functional and developmental integration lead to genetic integration which, in turn leads to evolutionary integration. Three examples of how developmental integration structures pleiotropy and morphological variation in non-human primate crania, artificially-modified human crania, and for the effects of individual genes on murine mandibular morphology are presented.

INTRODUCTION

Morphological integration is used here in its most general sense, referring to the connections or relationships among morphological elements. These connections can take many forms depending on the kinds of relationship being considered and the hierarchical levels at which they occur. Intra-individual morphological relationships involve functional and developmental integration while population-level morphological relationships involve genetic and evolutionary integration. As evolutionary morphologists we are interested in how individual-level functional and developmental relationships condition the inheritance and constrain or facilitate the evolution of morphological complexes. Theoretical models and related empirical tests seem to indicate that individual-level functional and developmental integration causes specific co-inheritance of morphological elements which, in turn, leads to their co-ordinate evolution (Cheverud, 1982, 1984, 1989, 1995, 1996). It is through its effects on the evolution of heritable variation that individual development affects population-level evolutionary processes.

LEVELS OF MORPHOLOGICAL INTEGRATION

Individual-level integration

Functional integration occurs when the functional interaction of morphological elements affects their joint performance. For example, the interplay of a honing mandibular premolar with its companion maxillary canine require their appropriate positioning in the lower and upper jaw along with a coordination of tooth size and shape.

Developmental integration occurs when morphological elements interact during their formation or are directed by a common external source. As an example of element interaction, the growth and maintenance of the mammalian mandibular coronoid process depends on the size and function of its associated musculature, the temporalis muscle (Moore, 1981). The muscle has an epigenetic effect on coronoid process morphology (Atchley and Hall, 1991). As an example of a common external source of developmental integration, circulating levels of growth hormone affect the growth rate of all mamma-

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lian limb long bones producing a developmental integration of these elements.

Both functional and developmental integration occur at the individual level. Furthermore, these two forms of individual-level integration are related because development can be viewed as dynamic function and functional integration in the adult is likely to be achieved through developmental integration.

Population-level integration

Genetic integration occurs when sets of morphological elements are inherited together, as a module, more or less independently of the other element sets or modules. Genetic integration is referred to the population rather than the individual level because genes are being considered here as units of inheritance rather than as units of physiological function. The physiological effects of genes relates to the developmental integration of morphological elements discussed above, while the co-inheritance of morphological elements involves passing them on from one generation to the next in concert, and is therefore a population-level process. Morphological elements may be inherited together due to pleiotropy, where a single gene affects multiple elements, or they may be inherited together due to linkage disequilibrium, where two genes, each affecting a single trait, tend to be inherited together (Falconer, 1989). The independent inheritance of morphological elements indicates either an absence of pleiotropy for genes affecting the elements in question or a balance between positive and negative pleiotropy (Cheverud, 1984). An example of genetic integration is that when individuals inherit a longer than average forelimb they are also likely to inherit a longer than average hindlimb (Leamy, 1977) indicating genetic integration of limb lengths. Genetic integration is due to the common effects of genes acting in epigenetic processes, such as those controlling the level of circulating growth hormone and the sensitivity of its target tissues. Thus, developmental integration structures genetic integration.

Evolutionary integration occurs when morphological elements evolve in a co-ordinated fashion. This may occur because the elements are inherited together or because they are selected together even though they are inherited independently (Felsenstein, 1988). We seek to understand why and how character complexes evolve and how their evolution constrains and/or facilitates adaptive responses to selection.

EVOLUTIONARY MODELS OF MORPHOLOGICAL INTEGRATION

Quantitative genetic models specify the relationships between the levels of morphological integration (Lande, 1980; Cheverud, 1982, 1984, 1989*a, b*) and generally indicate that patterns of developmental and functional integration cause genetic integration which, in turn, results in evolutionary integration.

The co-inheritance of morphological elements is specified by their genetic correlation or covariance. This is a population-level parameter specifying the strength of co-inheritance. The level and pattern of genetic correlation may evolve, as it depends both on genotypic effects and allele frequencies.

Lande (1980; Cheverud, 1984) showed that at equilibrium the pattern of genetic covariance (**G**), a quantitative expression of genetic integration, depends on the pattern of stabilizing selection (**W**) and the pattern of new variation and covariation produced by mutation (**U**),

$$\mathbf{G} = \mathbf{W}^{1/2}(\mathbf{W}^{-1/2}\mathbf{U}\mathbf{W}^{-1/2})^{1/2}\mathbf{W}^{1/2},$$

where **G** is the genetic variance/covariance matrix, **W** is the pattern of stabilizing selection on additive genetic, or heritable, values, and **U** is the mutation variance/covariance matrix. Generally, this means that the pattern of co-inheritance will evolve to match the pattern of stabilizing selection and the pattern of mutational effects.

If one assumes that mutation has uniform effects, so that each element has the same mutational variance and positive and negative pleiotropy cancel out, the genetic variance/covariance matrix is proportional to the matrix of stabilizing selection (Cheverud, 1984). The pattern of stabilizing selection reflects the functional and developmental relationships among morphological elements. It has small values for character combinations in which variation is not tolerated be-

cause deviations result in lack of fitness and large values for character combinations which differ only a little in their performance. For example, the maxillary and mandibular dentition must interdigitate properly to effectively crush food items and allow their digestion in the gut. This leads to a pattern of stabilizing selection in which the common length of the jaws is of perhaps less importance for performance than the interdigitation of the teeth. Within limits, long jaws and short jaws may not differ much in performance from optimal intermediate length jaws. However, individuals with poorly coordinated jaws (short mandible combined with a long maxilla and vice versa) would be less efficient in processing food leading to lower performance and fitness. Stabilizing selection can also be produced by developmental processes, the so-called internal selection discussed by Riedl (1977). In this situation the size, maturity, and position of one morphological element relative to others is necessary for proper development to occur (Hall, 1992). The equation specified above shows that the level of genetic correlation or integration will evolve to roughly match the functional association specified by stabilizing selection. This may involve either an increase or decrease of correlation level representing an integration or disintegration of the morphological elements depending on the ancestral state.

The pattern of morphological variation produced by mutation also can have an important effect on patterns of genetic integration. Keightley and Hill (1992) found that the rate of production of heritable variation in murine body weight may be as high as 1% of the environmental variance per generation making mutational effects on variance significant for evolution in natural systems. The pattern of mutational variance depends largely on the pattern of potential pleiotropic effects of loci physiologically affecting a set of characters. Little is known, or even imagined, about the evolution of mutational variation patterns which would allow their prediction. However, Wagner (1996) presents a model for the evolution of pleiotropy by differential epistasis. This model predicts that characters which are selected together for a common function will either evolve or retain pleiotropy at the loci affecting them while functionally unrelated characters will lose or never evolve pleiotropy at loci affecting them. The gene-based developmental system evolves so that there is pleiotropy for functionally and developmentally integrated characters and no pleiotropy for functionally and developmentally unrelated characters. Whether the presence of pleiotropy leads directly to genetic integration or only provides the raw variation molded by stabilizing selection depends on the balance of positive and negative pleiotropy produced at the locus in question. So quantitative genetic models show that functional and developmental integration at the individual level leads to genetic integration at the population level. It is in this way that development can free or constrain evolutionary change.

Genetic integration leads to evolutionary integration, or the co-evolution of morphological elements, because selection on any component of a genetically integrated complex will lead to the co-ordinated evolution of the whole complex (Falconer, 1989; Lande, 1979; Cheverud, 1982; 1984). The expected evolutionary change for any given character is the combination of the effects of direct selection on that character and the indirect effects of selection on all other genetically-correlated characters,

$$\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta}$$

where Δz is the vector of changes in means for the morphological characters, $\mathbf{G}$ is the genetic variance/covariance matrix specifying patterns of genetic integration, and $\boldsymbol{\beta}$ is the selection gradient, measuring the direct selection on each character (Lande, 1979; Lande and Arnold, 1983). Characters evolve together because they are inherited together. However, characters can also evolve together even when they are not inherited together if they are co-selected for participation in a common function (Felsenstein, 1988).

Interestingly, in Wagner's (1996) model for the evolution of pleiotropy, co-selected traits either retain or enhance pleiotropy at loci affecting them while pleiotropy for traits which are not co-selected either never

evolves or is eliminated by selection. The co-selection of traits leads to the evolution of an integrated developmental system, in which specific modules are either built-up from non-integrated parts or whittled down from uniformly integrated parts. The modular gene-based developmental system then produces a modular pattern of heritable variation, with genetic integration of functionally and developmentally-related traits, which, in turn, leads to integrated evolution of members of the same character complex and mosaic evolution of character complexes.

I will provide three examples of empirical studies of morphological integration which seek to discern the effects of functional and developmental integration on patterns of morphological variation.

EMPIRICAL EXAMPLES

Primate cranial morphology

I have investigated cranial morphological integration in two widely divergent primate groups, the papionins (macaques, baboons, and mangabeys) (Cheverud, 1982, 1989) and the tamarins (Cheverud, 1995, 1996). Studies of mammalian cranial development (Moore, 1981; Hanken and Hall, 1993; Cheverud, 1982, 1995) indicate that the skull is composed of several semiautonomous functional/developmental complexes. At the broadest scale, the skull is composed of three parts, the cranial vault, cranial base, and face. In order to function effectively these three cranial parts must be intact and connected to each other, the cranial base linking the vault and the face. Pathological breaks in cranial elements resulting in disconnection between the face and braincase can lead to extreme morphological distortion indicating the basic functional unity of the cranium. However, different parts of the skull are influenced by the growth of different organs and systemic hormonal factors.

The growth of the cranial vault depends almost entirely on the growth of the brain, especially the cerebrum and cerebellum. Brain growth occurs prenatally and neonatally in eutherian mammals but completes its growth soon after birth. In contrast, a majority of facial growth occurs after the brain has stopped growing. The hormonal and underlying genetic basis for early and later mammalian growth is independent, leading to a general independence of early and late growing organs (Sara *et al.*, 1981; Atchley *et al.*, 1984; Riska *et al.*, 1984; Riska and Atchley, 1985). Cheverud and Routman (unpublished data) have found a lack of pleiotropy at genes affecting early and later growth in mice. Thus, we may expect some lack of integration between the early-growing cranial vault and the late-growing face. The cranial base is affected both by brain growth, especially the brain stem, and the factors influencing later growth (Moore, 1981).

In both the papionins and the tamarins, genetic integration was discovered within the masticatory apparatus of the face and the cranial vault, so that developmentally integrated traits tended to be inherited together, in contrast to developmentally unrelated traits (Cheverud, 1982, 1995, 1996). Since the same general pattern of genetic integration occurs in both New and Old World Monkeys, it is likely to occur generally through the anthropoid primates (monkeys, apes, and humans). Furthermore, the developmental patterns producing genetic integration in primates are based on physiological phenomena which occur throughout eutherian mammals, so it is possible that the patterns of genetic integration described here are general for the entire taxon.

However, interesting exceptions may also exist. In primates, the external table of the cranial vault runs parallel to its inner table with only relatively small raised ridges for the attachment of muscles of mastication and head posture so that the external cranial vault is a reasonably close representation of the brain. However, in other large mammals the temporal lines and nuchal crests may greatly enlarge and intracranial sinuses form so that the external surface of the skull is more strongly influenced by muscle attachments than by brain growth. This may lead to external cranial vault measures being more closely integrated with facial structures than is typical in primates. Even so, internal vault measures may still form an integrated set, separate from the face. Also, primates have relatively much larger

brains and extended growth periods relative to other mammals (Martin, 1990). The modularization of the braincase and face may be less extreme in groups in which the brain does not grow to such a large relative size. If so, the modularization of braincase and face in primate evolution would be a consequence of prolonging the early growth period to larger brain and body size resulting in the relatively large primate brain. It would also be interesting to determine whether these patterns of cranial integration also apply to non-eutherian mammals which display early development of the masticatory apparatus relative to the brain (Smith, 1996).

Artificially modified human crania

Although the face and braincase are influenced by separate developmental systems, the necessity of maintaining a physically integrated cranium allows the early, prior growth of the braincase to act as a developmental constraint on facial growth. In effect, this represents an epigenetic effect of brain growth on facial morphology. This is illustrated by the effect of artificial modification of human cranial vault shape on facial morphology. At different times and places throughout the world various cultures have purposefully molded the cranial vaults of their children in order to produce an appealing appearance (Cheverud *et al.*, 1992; Kohn *et al.*, 1993). Two major classes of such modification are fronto-occipital flattening, where bands or boards are tied across the frontal and occipital bones resulting in a short, wide cranial vault, and annular modification, where bands are tied in a circular fashion around the cranial vault producing a long, narrow cranial vault.

The appliances are placed only on the cranial vault but also affect the growth of the cranial base and face. Opposing forms of modification result in opposing facial alterations. As shown diagramatically in Figure 1, with fronto-occipital modification anterior-posterior cranial vault growth is prevented by the appliance. This lack of anterior-posterior growth is compensated for by increased growth medial-laterally. The increased width of the cranial vault leads to an increased width of the cranial base. The

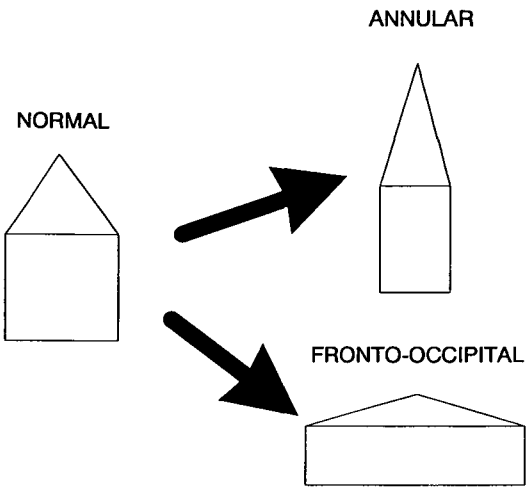

FIG. 1. Representation of the effects of annular and fronto-occipital artificial cranial vault modification on the cranial base and face. The rectangle represents the neurocranium while the triangle represents the face. The common edge represents the cranial base. With annular modification the vault becomes long and narrow, the cranial base narrows and the face protrudes while with fronto-occipital modification the vault becomes short and wide, the cranial base widens and the face retracts (Cheverud *et al.*, 1992; Kohn *et al.*, 1993).

increased width of the cranial base, in turn, leads to a wider, foreshortened face (Cheverud *et al.*, 1992) and mandible (Cheverud and Midkiff, 1992) because these parts must form a single bony structure with the cranial vault to insure adequate function. Likewise, annular modification prevents medial-lateral growth resulting in compensatory anterior-posterior growth and a long, narrow cranial vault (see Fig 1). The long, narrow vault leads to a narrow cranial base which, in turn, leads to a relatively long, narrow face (Kohn *et al.*, 1993).

Given these results, we expect that factors directly affecting brain width will affect cranial vault width and in turn, affect the relative length and width of the face. This should be true regardless of whether the factors involved are environmental in origin, as in the case described here, or genetic. The relationship between face and cranial vault morphology should be asymmetrical, in that factors strongly affecting facial morphology after the brain has ceased growth should not affect cranial

vault morphology, except for increasing the size of bony ridges for muscle attachment.

Murine mandibular morphology

The mandible is a complex bone composed of several different mesenchymal condensations and influenced by several semiautonoumous related organs. The horizontal ramus or alveolar portion of the mandible supports the teeth. Alveolar bone develops in response to dental development and is only maintained in the presence of teeth (Moore, 1981; Atchley and Hall, 1992; Hall, 1992). In contrast, the ascending ramus is site of insertion for several masticatory muscles, the temporalis on the coronoid process, the masseter and medial pterygoid on the lateral and medial sides of the angular process, and the lateral pterygoid on the condyloid process. The condyloid process also maintains articulation with the rest of the cranium. The initial development of these portions of the ascending ramus depends on density of mesenchymal condensations while their further development and maintenance depends on the presence and function of the associated musculature (Moore, 1981; Atchley and Hall, 1991). There is no known physiological or developmental link between dental and muscular development. Therefore, under the model of morphological integration described above, we expect genetic integration among alveolar features and among features of the muscular processes to be greater than the integration across these developmental units. This was found by Cheverud *et al.* (1991) in a study of mouse inbred strains. Based on Wagner's (1996) model for the evolution of pleiotropy, we expect developmentally-related traits to be affected by the same gene loci while developmentally unrelated traits are affected by different sets of loci.

In an F_2 intercross breeding experiment involving the cross of two inbred mouse strains, LG/J and SM/J, Cheverud, Routman, and Irshick (unpublished results) have mapped and measured the effects of 27 individual gene loci on mandibular morphology. The results indicate that about 20% of the loci have pleiotropic effects across the whole mandible, 40% of the loci affect the alveolus, and 40% affect the muscular processes of the ascending ramus. These results are consistent with the expectations of Wagner's (1996) model. Examples of gene loci with general, alveolar, and muscular process effects are shown in Figure 2.

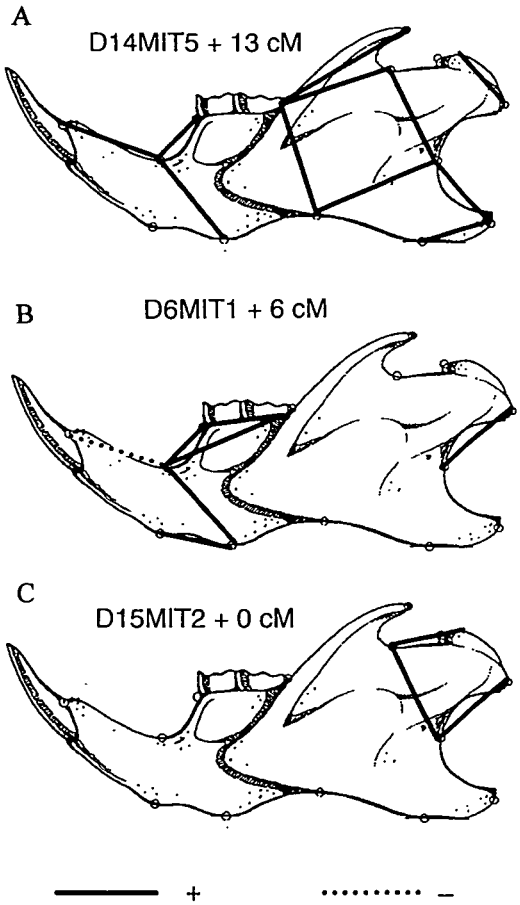

FIG. 2. Examples of individual gene loci affecting the mandible (Cheverud, Routman and Irshick, unpublished results). The locus is identified as being a certain distance (in centiMorgans) from a short sequence repeat locus. Line segments are drawn for all measurements significantly affected by the locus in question. Solid lines indicate positive effects for the allele originating from the LG/J strain while dashed lines indicate positive effects for the allele originating from the SM/J strain. (A) Effects throughout the mandible as in 20% of the loci. (B) A locus with alveolar effects, as in 40% of the loci. (C) A locus with effects on the ascending ramus, as in the remaining 40% of the loci.

CONCLUSIONS

Quantitative genetic theory predicts that functional and developmental relationships

between morphological elements will be represented in the genetic system (Wagner, 1996) and result in the specific co-inheritance of functionally and developmentally related traits, unrelated character complexes being inherited independently of one another. This facilitates the integrated evolution of functionally related characters while permitting the mosaic evolution of unrelated characters. Three examples were discussed in which functional and developmental relationships between morphological elements were used to predict or interpret patterns of morphological variation: a non-experimental analysis of population-level variation in cranial morphology, a natural experiment in the manipulation of cranial growth in artificially-modified human crania, and a genetic breeding experiment allowing the measurement of single gene effects. All three approaches illustrate the importance of morphological integration for understanding morphological evolution.

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REFERENCES

Atchley, W. R. and B. K. Hall. 1991. A model for the development and evolution of complex morphological structures. Biol. Rev., Camb. 66:101–157.

Atchley, W. R., B. Riska, L. Kohn, A. Plummer, and J. Rutledge. 1984. A quantitative genetic analysis of brain and body size associations, their origin and ontogeny: Data from mice. Evolution 38: 1165–1179.

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

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

Cheverud, J. M. 1989. Evolution of morphological integration. *In* H. Splechtna and H. Helge (eds.), *Trends in vertebrate morphology,* Proceedings of the 2nd International Symposium on Vertebrate Morphology, Fortschritte der Zoologie, Vol. 35, pp. 196–197. Fisher, New York.

Cheverud, J. M. 1995. Morphological integration in the saddle-back tamarin (*Saguinus fuscicollis*) cranium. Am. Nat. 145:63–89.

Cheverud, J. M. 1996 Comparison of genetic and phenotypic patterns of morphological variation in tamarin crania. J. Evol. Biol. 9. (In press)

Cheverud, J. M., S. E. Hartman, J. T. Richtsmeier, and W. R. Atchley. 1991. A quantitative genetic analysis of localized morphology in mandibles of inbred mice using finite element scaling analysis. J. Craniofac. Genet. Dev. Biol. 11:122–137.

Cheverud, J. M., L. Kohn, L. Konigsberg, and S. Leigh. 1992. Effects of fronto-occipital artificial cranial vault modification on the cranial base and face. Am. J. Phys. Anthropol. 88:323–345.

Cheverud, J. M. and J. E. Midkiff. 1992. Effects of fronto-occipital cranial reshaping on mandibular form. Am. J. Phys. Anthropol. 87:167–171.

Falconer, D. S. 1989. *Introduction to quantitative genetics.* Longman Press, London.

Felsenstein, J. 1988. Phylogenies and quantitative characters. Ann. Rev. Ecol. Syst. 19:445–471.

Hall, B. K. 1992. *Evolutionary developmental biology.* Chapman & Hall, New York.

Hanken, J. and B. K. Hall. 1993. *The skull,* Vol. 1, *Development.* University of Chicago Press, Chicago.

Keightley, P. D. and W. G. Hill. 1992. Quantitative genetic variation in body size of mice from new mutations. Genetics 131:693–700.

Kohn, L. A. P., S. Leigh, S. Jacobs, and J. M. Cheverud. 1993. Effects of annular cranial vault modification on the cranial base and face. Am. J. Phys. Anthropol. 90:147–168.

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

Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. Genetics 94:203–215.

Lande, R. and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Leamy, L. 1977. Genetic and environmental correlations of morphometric traits in randombred house mice. Evolution 31:357–369.

Martin, R. D. 1990. Primate origins and evolution. Princeton University Press, Princeton.

Moore, W. 1981. *The mammalian skull.* Cambridge University Press, Cambridge.

Riedl, R. 1977. *Order in living organisms.* Wiley, New York.

Riska, B. and W. R. Atchley. 1985. Genetics of growth predicts patterns of brain size evolution. Science 229:668–671.

Riska, B., W. R. Atchley, and J. J. Rutledge. 1984. A genetic analysis of targeted growth in mice. Genetics 107:79–101.

Sara, V., K. Hall, and L Wetterberg. 1981. Fetal brain growth: A proposed model for regulation by embryonic somatomedin. *In* M. Ritzen, A. Aperia, K. Hall, A. Larsson, A. Zetterberg, and A. Zettersrom (eds.), *Biology of normal human growth,* pp. 241–252. Raven, New York.

Smith, K. K. 1996. Integration of craniofacial structures during development in mammals. Amer. Zool. 36:70–79.

Wagner, G. P. 1996. Homology natural kinds and the evolution of modularity. Amer. Zool. 36:36–43.