The dynamics of developmental system drift in the gene network underlying wing polyphenism in ants: a mathematical model

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SUMMARY Understanding the complex interaction between genotype and phenotype is a major challenge of Evolutionary Developmental Biology. One important facet of this complex interaction has been called "Developmental System Drift" (DSD). DSD occurs when a similar phenotype, which is homologous across a group of related species, is produced by different genes or gene expression patterns in each of these related species. We constructed a mathematical model to explore the developmental and evolutionary dynamics of DSD in the gene network underlying wing polyphenism in ants. Wing polyphenism in ants is the ability of an embryo to develop into a winged queen or a wingless worker in response to an environmental cue. Although wing polyphenism is homologous across all ants, the gene network

INTRODUCTION

A major goal of Evolutionary Developmental Biology is to understand the black box between evolution of the phenotype and evolution of the genotype (Raff 1996; Hall 1999; Wilkins 2001; Hall and Olsen 2003). This has led researchers to the surprising discovery that similar (homologous) phenotypes may not always be encoded by similar (homologous) genes (de Beer 1971; Hall 1995; Abouheif et al. 1997; Abouheif 1999; Wray 1999; Wray and Abouheif 1999). Recent studies and reviews have revealed cases where a similar phenotype, that is homologous across a group of related species, may be produced by the expression of different, nonhomologous, genes in each of the related species (Eizinger and Sommer 1997; Gibson 2000; Ludwig et al. 2000; Abouheif and Wray 2002; Felix 2005). Sex determination, for example, is a homologous trait in closely related fly species, but the upstream genes in the pathway that control sex determination in these flies are different in different species (Marin and Baker 1998; Schutt and Nothinger 2000). This phenomenon has been called "Developmental System Drift" (DSD; True and

that underlies wing polyphenism has evolved. In winged ant castes, our simulations reproduced the conserved gene expression patterns observed in the network that controls wing development in holometabolous insects. In wingless ant castes, we simulated the suppression of wings by interrupting (up- or downregulating) the expression of genes in the network. Our simulations uncovered the existence of four groups of genes that have similar effects on target gene expression and growth. Although each group is comprised of genes occupying different positions in the network, their interruption produces vestigial discs that are similar in size and shape. The implications of our results for understanding the origin, evolution, and dissociation of the gene network underlying wing polyphenism in ants are discussed.

Haag 2001). Although comparative studies have established the pattern of DSD in a wide variety of taxa, the developmental and evolutionary mechanisms underlying DSD remain poorly understood (True and Haag 2001; Angelini and Kaufman 2005).

Computational and mathematical modeling of genetic regulatory networks permits exploration of the possible dynamic behaviors, and may reveal new genetic interactions (von Dassow et al. 2000; Jaeger et al. 2004; Perkins et al. 2006). Since the use of mathematical models have been helpful for understanding the mechanisms of pattern formation in development (Eldar et al. 2003; Kruse et al. 2004; Saha and Schaffer 2006), they may also be useful to study the mechanisms of DSD. In this article, we developed a mathematical model to investigate the divergence of the gene network underlying wing polyphenism in ants.

Developmental geneticists have elucidated the gene network that controls wing development in *Drosophila* (Fig. 1A; reviewed in Cohen 1993; Held 2002). This "wing-patterning" network has been evolutionarily conserved across the holometabolous insects (Diptera, Lepidoptera, Coleoptera,



Fig. 1. Conservation of the wing-patterning network in holometabolous insects. (A) The gene network that controls patterning and growth of wing imaginal discs in Drosophila melanogaster operates in three phases (I-III). First (I), a set of transcription factors and signaling molecules establish a cluster of approximately 20 ectodermal cells as precursors of both the leg and the wing imaginal discs. Second (II), a set of interacting genes then divides these cells into separate clusters that give rise to three pairs of leg discs and two pairs of wing imaginal discs. Third (III), a set of interacting transcription factors and signaling molecules pattern these wing discs, imparts to them a wing-specific identity, and activates downstream target genes that pattern the detailed structures of the wing. (B-G) Expression of several genes within this network is conserved in wing discs across holometabolous insects. (B) Representation of the conservation of engrailed expression in the posterior (P) and not in the anterior (A) compartment of Drosophi*la*, butterfly, beetle, and ant wing discs. (C-F) Representation of the conservation, between Drosophila and ant wing discs of: (C) wingless expression along the dorsal (D)-ventral (V) margin; (D) extradenticle expression in the hinge region; (E) scalloped expression in the wing pouch; (F) spalt expression along the AP axis marking the position of future wing veins; and (G) Ultrabithorax expression in the hindwing but not the forewing.

and Hymenoptera) for at least 325 Myr (Abouheif and Wray 2002). For example, the expression of *engrailed*, a transcription factor that plays a key role in specifying the posterior compartment of the wing imaginal disc, is conserved in the wing discs of flies, butterflies, beetles, and winged castes of ants (Fig. 1B; Carroll et al. 1994; Abouheif and Wray 2002; Tomoyasu et al. 2005). The expression of other genes within this network have also been remarkably conserved (Fig. 1, B–G), indicating a conservation of the function of these genes. The *Hox* gene, *Ultrabithorax*, is most likely an exception in that its expression is conserved (Fig. 1G), but its function has evolved to play new roles during wing development and evolution in holometabolous insects (Weatherbee et al. 1998, 1999; Tomoyasu et al. 2005).

Polyphenism is the ability of a single genome to give rise to alternative phenotypes in response to an environmental cue (Nijhout 1999, 2003). Wing polyphenism in ants is the ability of the wing-patterning network to give rise to queens with functional wings or workers that are wingless in response to environmental cues, such as temperature, photoperiod, or nutrition. Wing polyphenism, which evolved just once approximately 125 Ma, is homologous across all ant species. This is supported by two observations: (1) wing polyphenism is a nearly universal feature among the 12,000 known ant species (Hölldobler and Wilson 1990); and (2) the earliest known ant fossils possess wingless workers (Wilson 1987). Because wing polyphenism is homologous across all ant species, one might have predicted that the mechanisms through which wings are suppressed in the worker castes of all ant species would also be the same. However, Abouheif and Wray (2002) demonstrated that although expression of the wingpatterning network underlying wing polyphenism is conserved

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in the winged castes of four ant species, expression of this network is different in the wingless castes of these species. For example, *engrailed* is expressed in a conserved pattern in the posterior compartment of the wing disc in the winged castes of two ant species, Pheidole morrisi and Crematogaster lineolata (Fig. 1B; Abouheif and Wray 2002). In the wingless worker castes, however, engrailed is expressed in the posterior compartment in the vestigial forewing discs in P. morrisi, but is not expressed in the vestigial wing discs in C. lineolata. An interruption point is defined as a gene whose expression is modified (up- or downregulated) in the wing vestiges of wingless ant castes. Thus, the downregulation of engrailed expression in the workers of C. lineolata is an interruption point. This is an example of DSD because the expression of genes in the wing-patterning network is different in the worker castes of these two species even though wing polyphenism is the same.

Currently, there are at least three simple models to explain the evolution of the gene network underlying wing polyphenism in ants: natural selection, genetic drift, or a combination of both (Abouheif 2004). The first model, natural selection, could explain the divergence of the gene network if selection directly acts on many different genes in the wingpatterning network in different species, or if selection acts indirectly on many different genes in the network through selection on a correlated phenotype. The second model, genetic drift, could explain the divergence of the wing-patterning network if genes in the network do not play a functionally important role in repressing wing development, and the wing imaginal disc is simply eliminated through apoptosis in the last stages of development (Sameshima et al. 2004). Finally, the third model, a combination of natural selection and genetic drift, could explain the divergence of the wing-patterning network if the suppression of wings in the worker caste was mediated through a key regulatory gene. This key gene behaves as a control node in the gene network, in which genes upstream of the key regulator would evolve neutrally or by selection, as long as they do not perturb suppression of wings in workers. Because of its important role in maintaining the suppression of wings, the key regulatory gene would be under stabilizing selection.

Research on *Drosophila* wing development has uncovered a gene that has major effects on patterning and growth of wing discs called *brinker* (*brk*; Campbell and Tomlinson 1999; Minami et al. 1999; Jaźwińska et al. 2002). *brk* encodes a transcription factor that controls cell proliferation in the wing disc and regulates expression of downstream target genes, such as *spalt* (*sal*), that pattern detailed structures along the anterior–posterior (AP) axis of the wing (Fig. 1F; Campbell and Tomlinson 1999; Minami et al. 1999; Martín et al. 2004; Winter and Campbell 2004; Moser and Campbell 2005; Affolter and Basler 2007). There are several key observations in ants and experiments in *Drosophila* that point to *brk* as



Fig. 2. The AP subnetwork (module) within the wing-patterning network. An expanded representation of the AP subnetwork that controls patterning along the AP axis. mRNA (lower case letters) and proteins (first letter capitalized) are shown separately for the *dpp* signaling molecule and the genes encoding the receptors (*ptc* and *tkv*), because only in these cases the mRNA expression pattern is expected to differ from that of the protein. Arrows represent activation and truncated lines represent repression.

the key gene that mediates the suppression of wings in the wingless worker castes in ants. First, comparative studies in ants have shown that in all ant species examined to date (Abouheif and Wray 2002; Bowsher et al. 2007), interruption points are always restricted to the subnetwork that patterns the AP axis of the wing disc (Fig. 2), and never in the subnetwork responsible for dorsal-ventral (DV) patterning. This suggests that only genes in the AP subnetwork (Fig. 2), such as *brk*, mediate wing suppression in wingless workers. Second, genetic experiments in Drosophila have shown that brk is a key regulator of cell proliferation and growth in the Drosophila wing. Downregulation of brk in Drosophila wing imaginal discs results in very large wing discs and consequently overinflated adult wings, whereas upregulation of brk results in very small wing discs (reminiscent of vestigial wing discs in worker ants) and an almost complete loss of wings in adult flies (Martín et al. 2004). brk mediates this effect on growth in a concentration-dependent manner, in that the size of wing discs and consequently the size of the adult wing, are inversely correlated to the concentration of brk that is expressed in the developing wing disc (Martín et al. 2004). Finally, brk has a dominant negative effect on growth when overexpressed together with other genes that promote growth such as *decapentaplegic (dpp)* (Lecuit et al. 1996; Martín et al. 2004). These experiments have demonstrated that *dpp*-dependent regulation of growth is only indirect and dependent on brk, which support the role of brk as the principal regulator of growth in wing discs (Martín

et al. 2004). Together, these observations and experiments suggest that brk is a key node in the wing-patterning network that mediates DSD in the gene network underlying wing polyphenism in ants, and that DSD may be driven by a combination of selection and drift. We developed a mathematical model to explore this idea, as well as to reveal the developmental and evolutionary dynamics of DSD in the gene network underlying wing polyphenism in ants.

THE MODEL

Modeling the AP wing-patterning network in winged and wingless ant castes

A simplified version of the gene network that controls patterning and growth along the AP axis in wing discs is depicted in Fig. 2. We focused exclusively on the AP axis because gene networks that pattern the AP and DV axes of the wing disc behave as independent modules (Carroll et al. 2005), and comparative studies in ants have shown that the AP network has been the primary target of evolutionary change in wingless ant castes (Abouheif and Wray 2002; Bowsher et al. 2007). Our model is restricted to one spatial dimension along the center of the AP axis of the wing disc during the third larval instar. Gene names that represent mRNAs were italicized and lower cased, whereas names that represent proteins were nonitalicized and the first letter was capitalized. For example, "dpp" represents the mRNA form and "Dpp" the protein. Because total protein concentrations are linearly correlated to mRNA concentrations (Gilman and Arkin 2002), we only considered the dynamics of both mRNA and protein concentrations when the protein was expected to have a different spatial expression profile from its gene, as is the case for proteins that diffuse or form complexes with other proteins. We used a set of reaction-diffusion equations to simulate the concentrations of genes (mRNA) and proteins. These equations are similar to those in previous studies that modeled patterning in developmental systems (von Dassow et al. 2000; Lander et al. 2002; Eldar et al. 2003). The standard form of these equations is

$$(A) \quad \frac{\partial[g]}{\partial t} = \alpha_g H([F]) - \beta_g[g],$$

$$(B) \quad \frac{\partial[G]}{\partial t} = D_G \frac{\partial^2[G]}{\partial x^2} + T_G[g] - \gamma_{F_G}([F])([G]) - \beta_G[G],$$

$$(C) \quad \frac{\partial([F_G])}{\partial t} = \gamma_{F_G}([F])([G]) - \beta_{F_G}([F_G]),$$

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where [g], [F], [G], and $[F_G]$ represent the concentration of g, F, G, and F_G, respectively. Equation (A) represents the control of the mRNA synthesis of gene g as a function of [F]. Transcription of gene g is modeled using Hill-type functions H([F]). The sigmoidal shape of these functions implies that the

rate of transcription of a gene depends on the expression of its activator (or the absence of its repressor), but saturates at the maximal transcription rate (α_g) due to limited access to DNA. In equations of the form (A), H([F]) can take one of two forms (depending if F is an activator or repressor):

$$H([F]) = \frac{[F]^n}{k^n + [F]^n} (\text{ if } F \text{ is an activator}), \text{ or}$$
$$H([F]) = \frac{k^n}{k^n + [F]^n} (\text{ if } F \text{ is an activator}),$$

where *n* is the cooperativity (or Hill) coefficient and is a measure of the steepness of the sigmoidal curve. *k* is the concentration of activator (repressor) required to activate (repress) transcription at half of the maximal rate α_g . We assumed that chemical species *g* undergoes linear degradation at a rate β_g .

Equations (B) and (C) model the concentration of protein G and protein complex F_G, respectively. The first term in equation (B) models diffusion in a one-dimensional domain (Fick's law), where D_G is the diffusion coefficient, and T_G is the rate of protein translation, which was assumed to be linearly proportional to the corresponding mRNA concentration. Formation of protein complexes F_G is modeled using a law of mass action in which the rate of the reaction $F+G \rightarrow F_G$ is proportional to the constant of proportionality is denoted by γ_{F_G} . As in (A), we assumed that G and F_G undergo linear degradation, and we used β_G and β_{F_G} to denote the degradation rate of G and F_G, respectively.

We used equations of the form (A), (B) or (C) to model the concentration of each of the gene products in Fig. 2 based on their network interactions. Engrailed (En) is expressed constantly throughout the third larval instar at high levels in the posterior compartment (Hama et al. 1990). We therefore set $[\text{En}](x, t) = 1 \ \mu\text{M}$ for $x > \frac{L}{2}$ (posterior compartment) and zero otherwise for all times. Here, *L* is the size of the disc along the AP axis, which was assumed to be constant (400 µm) throughout the duration of patterning.

Patterning along the AP axis is organized by two morphogens, *hedgehog* (*hh*) and *dpp*. *hh* transcription is activated by En in every posterior cell of the disc and a fragment of the Hh protein diffuses short-range into the anterior compartment. Extracellular Hh is sequestered upon binding to its receptor Patched (Ptc) and the Hh_Ptc complex is targeted for degradation. Thus, the concentration of Hh was modeled as

$$\frac{\partial [\text{Hh}]}{\partial t} = D_{\text{Hh}} \frac{\partial^2 [\text{Hh}]}{\partial x^2} + \alpha_{\text{Hh}} [\text{En}](x) - \gamma_{\text{Hh}_\text{Ptc}} [\text{Hh}] [\text{Ptc}] - \beta_{\text{Hh}} [\text{Hh}].$$
(1)

ptc is ubiquitously present at low levels in every cell of the anterior compartment and is activated transcriptionally by medium to high levels of Hh signaling (Vervoort 2000). This

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was expressed as

$$\frac{\partial [ptc]}{\partial t} = \alpha_{ptc0}(x) + \alpha_{ptc} \frac{[\text{pSmo}]^n}{k_{ptc}^n + [\text{pSmo}]^n} - \beta_{ptc}[ptc], \qquad (2)$$

where $\alpha_{ptc0}(x) = \text{constant}$ for $x \leq \frac{L}{2}$ (anterior compartment) and zero otherwise. *ptc* is then translated to Ptc and then translocated to the plasma membrane where it binds extracellular Hh. The formation of the Hh_Ptc complex is essential for the spatial control of Hh signaling. The concentrations of Ptc and Hh_Ptc were modeled as

$$\frac{\partial [\text{Ptc}]}{\partial t} = T_{\text{Ptc}}[ptc] - \gamma_{\text{Hh}_\text{Ptc}}[\text{Hh}][\text{Ptc}] - \beta_{\text{Ptc}}[Ptc] \qquad (3)$$

and

$$\frac{\partial [\text{Hh}_{Ptc}]}{\partial t} = \gamma_{\text{Hh}_{Ptc}} [\text{Hh}] [\text{Ptc}] - \beta_{\text{Hh}_{Ptc}} [\text{Hh}_{Ptc}].$$
(4)

The Hh signaling transducer protein Smoothened (Smo) is constitutively present in every cell of the anterior compartment (Held 2002). In the absence of Hh, Smo is found in an unphosphorylated form and cannot transduce Hh signaling. However, in the presence of Hh, the Hh_Ptc complex relieves Ptc repression and allows Smo phosphorylation. Since Smo is a transmembrane protein, there should be a maximum occupancy of Smo in the plasma membrane. We modeled this by assuming that the total levels of Smo ([Smo_{Total}]) remain constant ([Smo_{Total}] = [Smo]+[pSmo], where pSmo represents the phosphorylated form of Smo). How pSmo is regulated remains unclear, but appears to depend on the ratio of occupied (Hh_Ptc) to unoccupied receptors (Ptc) (Casali and Struhl 2004). We modeled this process as

$$\frac{\partial [\mathbf{pSmo}]}{\partial t} = \alpha_{\mathbf{pSmo}}(x) \frac{\left(\frac{[\mathbf{Hh}-\mathbf{Ptc}]}{[\mathbf{Ptc}]}\right)^{m}}{k_{\mathbf{pSmo}}^{m} + \left(\frac{[\mathbf{Hh}-\mathbf{Ptc}]}{[\mathbf{Ptc}]}\right)^{m}} - \beta_{\mathbf{pSmo}}[\mathbf{pSmo}], \tag{5}$$

with $\alpha_{pSmo}(x) = \text{constant for } x \le \frac{L}{2}$ (anterior compartment) and zero otherwise.

dpp expression is an output of Hh signaling. It is expressed in a broader domain than *ptc* and hence is activated by lower levels of pSmo (Vervoort 2000). Thus, the equation governing *dpp* expression is similar to equation (2), and was modeled as

$$\frac{\partial [dpp]}{\partial t} = \alpha_{dpp} \frac{[\text{pSmo}]^n}{k_{dpp}^n + [\text{pSmo}]^n} - \beta_{dpp} [dpp]. \tag{6}$$

Although *dpp* expression is confined to a 10–12 cell stripe anterior to the AP boundary (Casali and Struhl 2004), the Dpp protein acts as a long-range signal that patterns the whole wing disc (Nellen et al. 1994). Although the mechanism of Dpp transport remains controversial (Lander et al. 2002; Kruse et al. 2004), we used simple diffusion to model its expression. Given that Dpp diffusion is limited by binding to its receptor Thickveins (Tkv), [Dpp] dynamics was modeled as

$$\frac{\partial [\mathbf{Dpp}]}{\partial t} = D_{\mathbf{Dpp}} \frac{\partial^2 [\mathbf{Dpp}]}{\partial x^2} + T_{\mathbf{Dpp}} [dpp] - \gamma_{\mathbf{Dpp}-\mathbf{Tkv}} [\mathbf{Dpp}] [\mathbf{Tkv}] - \beta_{\mathbf{Dpp}} [\mathbf{Dpp}].$$
(7)

thickveins (tkv) is transcribed ubiquitously in both anterior and posterior cells, but its expression is higher in posterior cells, presumably through activation of En and downregulation in the Hh signaling region (Tabata 2001). This was modeled as

$$\frac{\partial [tkv]}{\partial t} = \alpha_{tkvP} [\text{En}](x) + \alpha_{tkv0} \frac{k_{tkv}^n}{k_{tkv}^n + [\text{pSmo}]^n} - \beta_{tkv} [tkv].$$
(8)

Translation of *tkv* to Tkv and binding of Tkv to Dpp leads to the formation of the Dpp_Tkv complex:

$$\frac{\partial [\text{Tkv}]}{\partial t} = T_{\text{Tkv}}[tkv] - \gamma_{\text{Dpp}_{\text{Tkv}}}[\text{Dpp}][\text{Tkv}] - \beta_{\text{Tkv}}[\text{Tkv}] \quad (9)$$

and

$$\frac{\partial [Dpp_Tkv]}{\partial t} = \gamma_{Dpp_Tkv} [Dpp] [Tkv] - \beta_{Dpp_Tkv} [Dpp_Tkv].$$
(10)

Upon binding to Dpp, the Dpp_Tkv complex induces phosphorylation of Mothers against dpp (Mad), which acts directly as a transcription activator of Dpp target genes. We assumed that Mad is abundantly expressed in the disc and its rate of phosphorylation is proportional to [Dpp_Tkv]. This assumption, in principle, may allow for the unlimited expression of phosphorylated Mad (pMad), but this process is self-limited by repression of a pMad target gene called *Daugthers against dpp (Dad)*. This was modeled as

$$\frac{\partial [pMad]}{\partial t} = \alpha_{pMad} \frac{k_{pMad}^{n}}{k_{pMad}^{n} + [Dad]^{n}} [Dpp_{-}Tkv] - \beta_{pMad} [pMad].$$
(11)

Although pMad activates target genes directly, the most important role of pMad seems to be to repress the expression of *brk*, which gives rise to an opposing gradient of [Brk] that directly represses the Dpp target genes (Müller et al. 2003). Recent studies have proposed that self-regulation of *brk* is necessary to generate a graded [Brk] gradient (Moser and Campbell 2005). We did not include this interaction in our model because it remains unclear whether *brk* represses itself directly or indirectly through Brk downregulation of *Dad*. Thus, [Brk] was modeled as

$$\frac{\partial [\text{Brk}]}{\partial t} = \alpha_{\text{Brk}} \frac{k_{\text{Brk}}^n}{k_{\text{Brk}}^n + [\text{pMad}]^n} - \beta_{\text{Brk}} [\text{Brk}].$$
(12)

The Dpp target genes *sal*, *optomotor-blind* (*omb*), and *Dad* are activated at high, medium, and low thresholds of pMad expression, respectively. However, these genes are also inhibited by differential concentrations of Brk. Because the differential control of these genes depends on two inputs ([pMad] and [Brk]), we used modified versions of Hill

functions to model these interactions as

$$\frac{\partial [sal]}{\partial t} = \alpha_{sal} \left[\frac{k_{salR}^{j}}{k_{salR}^{j} + [\mathbf{Brk}]^{j}} \right] \left[\frac{[\mathbf{pMad}]^{n}}{k_{salA}^{n} + [\mathbf{pMad}]^{n}} \right] \\ - \beta_{sal} [sal], \tag{13}$$

$$\frac{\partial [omb]}{\partial t} = \frac{\alpha_{omb} [pMad]^n}{k_{omb}^n + [pMad]^n + [Brk]^n} - \beta_{omb} [omb], \quad (14)$$

and

$$\frac{\partial [\text{Dad}]}{\partial t} = \frac{\alpha_{\text{Dad}} [\text{pMad}]^n}{k_{\text{Dad}}^n + [\text{pMad}]^n + [\text{Brk}]^n} - \beta_{\text{Dad}} [\text{Dad}].$$
(15)

The first term in the right-hand side of equation (13) is the product of two independent Hill functions, and represents activation by pMad and repression by Brk. This follows from the observation that pMad and Brk binding sites do not overlap in the sal enhancer, and thus, their regulation may be independent (Barrio and de Celis 2004). In equation (13), when both pMad and Brk are present, the repressor will have a dominant effect. This is consistent with experimental data (Moser and Campbell 2005). Recent studies suggest that Dpp target genes may be regulated by binding competition of pMad and Brk (Kirkpatrick et al. 2001; Rushlow et al. 2001; Saller and Bienz 2001). Although the pMad and Brk binding sites in omb and Dad have not yet been identified, we employed a single term in equations (14) and (15) to model competition of pMad and Brk to the enhancer as in other theoretical studies (Ingolia 2004).

Simulating gene expression of the wingpatterning network in winged and wingless ant castes

Imaginal discs are divided into compartments very early in development. Expression of engrailed in the posterior compartment, and basal expression of *ptc* in the anterior compartment can be regarded as initial conditions of the system since normal AP patterning is rescued after hh expression is interrupted for 24 h (Strigini and Cohen 1997). On the other hand, because imaginal disc development is autonomous, we assumed that no net flux of signaling molecules is transported across the boundary of the disc (see supplementary Appendix S1 and Fig. S1 for details on initial and boundary conditions). We simulated gene expression patterns in winged ant castes by numerically solving the system of equations (1)-(15) in Matlab (supplementary Appendix S1). We extracted parameters for equations (1)-(15) from the literature, or estimated them from experimental observations (supplementary Appendix S2). In wingless ant castes, we simulated the interruption of the AP patterning network by varying (one at a time) particular parameters in equations (1)–(15). We assumed that the interruption of the AP patterning network is caused by environmental and epigenetic regulation. The rate of production represented by the parameters α_{Hh} , γ_{Hh_Ptc} , α_{dpp} , α_{pSmo} , and α_{pMad} was independently reduced (downregulated) 10- and five-fold, whereas the parameters α_{ptc} , γ_{Dpp_Tkv} , α_{tkv} , α_{Brk} , and α_{Dad} were independently increased (upregulated) 10- and 5-fold from their original values (Table S1, supplementary Appendix S2). We then numerically solved Equations (1)–(15) (with all other parameters in Table S1, supplementary Appendix S2 held constant).

Modeling growth of wing discs in winged and wingless ant castes based on the differential regulation of Brk

Gradient formation along the AP axis in the wing disc reaches a steady-state long before completion of growth (Lecuit et al. 1996). Our preliminary simulations approximately attained a steady-state in 6–8 h, in agreement with experiments, which showed that the Dpp gradient is formed in less than 4 h (Teleman and Cohen 2000). In contrast, cells in the *Drosophila* wing disc divide uniformly approximately every 8 h (González-Gaitán et al. 1994). These observations suggest that the time-scales for patterning and growth are different, and thus, growth is insensitive to the transient dynamics of gene expression.

To model growth, we assumed that steady-state levels of Brk determine the local rate of cell proliferation. We called the steady-state expression of Brk at position x in the winged castes $Brk_Q(x)$, and in the wingless castes $Brk_W(x)$. We assumed that the steady-state levels of Brk set the required levels to proliferate every 8 h in a space-dependent manner along the AP axis, where the proliferation rate decreases with increases in $[Brk_Q](x)$.

We compared the level of $[Brk_W](x)$ in each cell of a worker $([Brk]_W)$ to the Brk concentration expected in a queen $([Brk]_Q)$ at the same position. Since the actual compartment sizes of the disc at the beginning of the third larval instar may differ between winged and wingless castes, it was convenient to express the position *x* in units relative to the size of each compartment. We defined the growth factor function, $G_T(x)$, as the average number of times that a cell in the position *x*, proliferates in a determined period of time *T*. We assumed

$$\mathbf{G}_T(x) = C\Omega([\mathbf{Brk}]_{\mathbf{Q}}(x) - [\mathbf{Brk}]_{\mathbf{W}}(x)), \quad (16)$$

where $C = \frac{R}{T} = \text{constant}$, with *R* being the uniform proliferation time (8 h) and *T* the observation time from the beginning of the third larval instar. Ω is a positive function that satisfies $\Omega(0) = 1$, which requires uniform proliferation at a growth factor *C* when $[\text{Brk}]_Q(x) = [\text{Brk}]_W(x)$. Because there are maximal physiological limits to the rates at which mitosis can occur, we assumed that cell proliferation saturates to a maximal rate when the difference $[\text{Brk}]_Q(x)-[\text{Brk}]_W(x)$ is too

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large. Thus, we proposed that Ω might be modeled as

$$\Omega([\mathbf{Brk}]_{\mathbf{Q}}, [\mathbf{Brk}]_{\mathbf{W}}) = \left[1 + \frac{[\mathbf{Brk}]_{\mathbf{Q}} - [\mathbf{Brk}]_{\mathbf{W}}}{\kappa + ([\mathbf{Brk}]_{\mathbf{Q}} - [\mathbf{Brk}]_{\mathbf{W}})}\right]^{p}, \quad (17)$$

where κ and *p* are parameters that were estimated to fit the function in equation (17) to the available experimental data (Lecuit et al. 1996; Martín et al. 2004; see supplementary Appendix S2). κ is a measure of how precise a cell can sense differences in levels of Brk, whereas *p* determines the maximal proliferation rate: $\Omega_{\text{Max}} = 2^p$. Once the growth factor function G(x) was determined, the size of the disc (after *T* hours of the beginning of the last larval instar) was computed by integrating over the AP axis. This is

$$L_T = \int_{0}^{L} 2^{G(x)} \mathrm{d}x.$$
 (18)

The factor of 2 in equation (18) accounts for proliferation of daughter cells, which were assumed to proliferate at the same rate and eventually reach the same size as parent cells.

To simulate the effects of particular interruption points on the growth of wing discs in worker castes, we modified the same parameters listed above 10-fold, 5-fold and 2-fold, and solved numerically using equations (1)–(15) (with all other parameters in Table S1, supplementary Appendix S2 held constant). Finally, we used T = 24 h in equations (16)–(17) to simulate disc growth and (18) to estimate the size of vestigial wing discs after 1 day after the beginning of the last larval instar.

RESULTS

Simulation of the wing-patterning network generated conserved patterns of gene expression in winged ant castes

Simulations based on our mathematical model generated numerical profiles that represent expression patterns of genes from the wing-patterning network. The observed *Drosophila* gene expression patterns and intensity plots for three genes that pattern the AP axis in the wing-patterning network are shown in Fig. 3: pMad (Fig. 3, C and D), Brk (Fig. 3, F and G), and *sal* (Fig. 3, I and J). We compared the simulated profiles, obtained by numerically solving equations (1)–(15) using the parameters in supplementary Table S1, Appendix S2 with observed expression data from *Drosophila*. We assumed that the simulated profiles also reflect conservation of gene expression in winged ant castes.

pMad is a transcriptional activator, and is the main readout of the Dpp signaling pathway. pMad is expressed in regions where Dpp signaling is transduced to activate downstream target genes such as *sal*, *omb* and *Dad*. The numerical profile for [pMad] (Fig. 3E) shows good qualitative fit to the observed expression pattern and intensity plot (Fig. 3, C and D) in that it accurately generates two peaks of expression along the AP axis. brk, the key gene that regulates expression of target genes from the Dpp signaling pathway, and controls growth in the wing disc, is expressed in the lateral regions of the disc. The numerical profile for [Brk] (Fig. 3H) shows a good qualitative fit to the observed expression profile and intensity plot (Fig. 3, F and G). The downstream target gene sal is expressed as a broad stripe around the AP boundary, where it will serve to position the future development of wing veins. The numerical profile for [sal] (Fig. 3K) shows a good qualitative fit to the observed expression pattern (Fig. 3I). Although it is not evident in the intensity plot of sal (Fig. 3J), our simulations reproduced the drop of sal expression commonly observed at the AP boundary (Tabata and Takei 2004). Finally, our simulations reproduced the steady-state expression patterns of other genes in the wing-patterning network (Fig. S2).

Simulation of interruption points in wingless ant castes showed that they are unequal in their ability to affect patterning and target gene expression

Depending on their position in the wing-patterning network (Fig. 2), genes may be positive or negative regulators of *brk*. Positive regulators of *brk* antagonize cell proliferation, whereas negative regulators promote cell proliferation and disc growth. Thus, it is possible to produce small vestigial wing discs in the workers by either downregulating the negative regulators of *brk* or upregulating the positive regulators of *brk*. Based on network interactions in Fig. 2, one might have predicted that the majority of genes would be negative regulators of *brk*. Our results show, however, that this is not the case. Upregulation of *ptc*, *tkv* or Dpp-Tkv leads to the upregulation of Brk (Fig. 4, B, E, and H). This could not have been concluded from simple inspection of the schematic interactions in Fig. 2.

Furthermore, different interruption points in the wingpatterning network have an unequal effect on the expression of target genes, such as *sal*. One would have predicted that the interruption of genes that directly or indirectly derepress *brk* would significantly reduce, if not completely abolish, the expression of the downstream target genes regulated by *brk*. However, the only interruption points that significantly reduced or abolished expression of *sal* were the downregulation of pSmo (Fig. 51), pMad (Fig. 50), *dpp* (10-fold only; Fig. 5L), and upregulation of Brk (Fig. 4L). This result is consistent with *Drosophila* mutants where *dpp* levels are reduced (Teleman and Cohen 2000), or *brk* is overexpressed (Campbell and Tomlinson 1999; Moser and Campbell 2005). Other interruption points, such as the downregulation of *Hh* (Fig. 5C), Hh Ptc (Fig. 5F), and upregulation of *ptc* (Fig. 4C)



Fig. 3. Comparison of experimental and simulated gene expression profiles. Gene expression patterns and intensity profiles of wild-type, third instar, Drosophila wing discs. (A, B) A diagram demonstrating how observed expression profiles are compared to simulated ones. (A) The wing pouch (box indicated by the dashed line) of Drosophila third larval discs along the AP axis is approximately 400 µm long. (B) Simulated profiles are one-dimensional representations of the gene concentration along the AP axis. (C, F, I) Protein expression using immunohistochemistry for pMad (C) and Brk (F), and mRNA expression using in situ hybridization for sal (I). (D, G, J) Intensity profiles generated from the experimental data. (E, H, K) Steady-state simulated profiles obtained from the mathematical model for pMad (E), Brk (H), and sal (K). Expression levels are quantified in arbitrary units. For comparison purposes, dotted lines limit the area in the observed profiles that is being simulated. In all panels and figures throughout the article, anterior is to the left. Observed expression patterns (C, F, I) and intensity profiles (D, G, J) are reprinted from Moser and Campbell, © 2005, with permission from Elsevier.

cause a slight reduction of *sal* expression, such that a fairly broad stripe of *sal* expression remains visible along the AP axis. Upregulation of *tkv* (Fig. 4F) causes a reduction of the *sal* expression domain along the AP axis. Although this result appeared counterintuitive, it is consistent with *Drosophila* mutants, in which Tkv (Crickmore and Mann 2006) and Dpp_Tkv (Martín et al. 2004) was overexpressed in the wing disc. Finally, *Dad*, a downstream target gene of *brk*, causes a large, but not complete, reduction in *sal* expression (Fig. 4O). This is the only result in which our model is inconsistent with *Drosophila* mutants; upregulation of Dad should cause reduction of *sal* expression through its repressive effects on pMad, and consequent increase of Brk expression (Martín et al. 2004). However, Fig. 4N and O shows that although *sal* expression was reduced, Brk expression remains unchanged. Because the repressive effects of *Dad* on pMad are not well understood (Tsuneizumi et al. 1997), it is possible that the parameters estimated for this interaction may be inaccurate.



Fig.4. Effects on AP patterning due to interruption of genes that antagonize growth of wing discs. Simulated gene expression profiles of pMad, Brk and sal at steady-state due to upregulation of ptc (A-C), tkv (D-F), Dpp_Tkv (G-I), Brk (J-L) and Dad (M-O). Solid black lines indicate that synthesis rates for these genes were independently increased (Δ) 5-fold, whereas the dashed black lines indicate that they were independently increased 10-fold. Solid gray lines indicate expression pattern in the winged caste. All panels are plotted in the same scale. Concentration of gene products (vertical axis) is expressed in units of μ M. Position along the AP axis in the disc is expressed in units of µm. At the bottom of each column, there is a relative scale bar centered on the AP boundary. This scale bar indicates the relative position with respect to each compartment to make comparison of our predictions and results easier in other species in which the corresponding physical location may be unknown.



Fig. 5. Effects on AP patterning due to interruption of genes that promote growth of wing discs. Simulated gene expression patterns of pMad, Brk and *sal* at steady-state due to downregulation of Hh (A–C), Hh_Ptc (D–F), pSmo (G–I), *dpp* (J–L) and pMad (M–O). Solid black lines indicate that synthesis rates for these genes were independently decreased (∇) 5-fold, whereas the dashed black lines indicate that they were independently decreased 10-fold. Solid gray lines indicate expression pattern in the winged caste. All panels are plotted in the same scale. Concentration of gene products (vertical axis) is expressed in units of μ M. Position along the AP axis in the disc is expressed in units of μ m. At the bottom of each column, there is a relative scale bar centered on the AP boundary. This scale bar indicates the relative position with respect to each compartment to make comparison of our predictions and results easier in other species in which the corresponding physical location may be unknown.

Interestingly, however, only a slight change is observed in Brk expression when Dad was upregulated 25-fold or even 50-fold (data not shown), indicating that Brk expression may be potentially robust to changes in *Dad* expression. Together, these results suggest that selection for the suppression of wings in the wingless worker caste would favor interruption points that significantly reduce or abolish *sal* expression, such as pSmo, pMad, *dpp* and Brk.

Simulation of interruption points in wingless ant castes showed that there are "groups" of interruption points with similar effects on growth

Figure 6 reveals a striking parallel between the consequences of an interruption point on patterning and growth in the discs of wingless ant castes. Genes (pSmo, dpp, pMad and Brk) that significantly reduce or abolish the expression of the target gene sal, also significantly reduce growth of wing discs in the wingless worker castes. Visualizing the final size of wing discs in the worker castes revealed four groups of genes in the wingpatterning network that have similar effects on the reduction of target gene expression and growth in wing discs. The first group (group I) of interruption points, as mentioned above, significantly reduce the size of discs in wingless workers relative to the queen (pSmo, dpp, pMad and Brk; average final size is about 15%), whereas interruption of a second group (group II) of genes (Hh, ptc, Hh_Ptc) produce discs that are about 30% that of the queen disc. A third group (group III) is the interruption of Dad, where the discs are larger than in group II, and are about 50% smaller relative to the queens. Finally, a fourth group (group IV) of interruption points (tkv and Dpp Tkv) actually produce wing discs that are roughly the same or slightly larger relative to wing discs in the queen.

Figure 6 also reveals that the interruption of genes in different positions in the network produce vestigial discs that are similar in size and shape. For example, a wing disc that is about six times smaller than a queen disc can be achieved by a 2-fold upregulation of Brk or a 10-fold downregulation of Hh. Although there appears to be little correlation between the position of interruption points upstream of brk and the final size of a disc, the final size of a disc in the wingless castes of different ant species will always be highly correlated to the levels of Brk expression. Furthermore, Fig. 6 shows that the degree to which 2-fold, 5-fold and 10-fold up- or downregulation of genes affects the growth of the disc depends on the group that interruption points belong to. For example, there is little difference between the final size of the disc achieved through 2-fold, 5-fold and 10-fold downregulation of *dpp* (group I). In contrast, there is a large effect observed between 2-fold, 5-fold and 10-fold downregulation of Hh Ptc (group II). The only exception is pSmo in which there is a large qualitative jump between a 2-fold and a 5-fold or



Fig. 6. Effects of interruption points on disc growth and final size. The final size of the disc in the winged queen caste is indicated by the black bar, while the final size of discs obtained by decreasing (∇) or increasing (Δ) by 2-fold (black bars), 5-fold (gray bars) and 10-fold (light gray bars) the values of the synthesis rates. The vertical dashed line indicates the position of the disc along the AP axis. Interruption points are classified into four groups (groups I–IV) according to their effects on disc growth. The numbers beside each bar indicate the percentage by which the discs in the workers are smaller than that of the queen.

10-fold downregulation of pSmo. This indicates that there may be a buffering capacity in some genes to environmental or genetic perturbations over and above the adaptive environmental cues expected by the developing ant larvae.

Finally, the shape of wing discs in wingless ant castes is not symmetrical along the AP axis. Interruption of most genes causes asymmetrical growth along the AP axis of the disc, which favors the anterior compartment. This is most likely

due to the observation that cells in each compartment do not intermix once they have acquired their AP identity, and that the expression of the *dpp* domain is biased toward the anterior compartment. Indeed, there exist vestigial wing discs in worker castes, such as those of *C. lineolata* (Abouheif and Wray 2002), that show a high degree of asymmetry along the AP axis.

Prediction of interruption points in the vestigial wing discs in wingless ant castes

The simulations which assessed the effects of particular interruption points on growth in Fig. 6, may be used to predict which group will be interrupted in the vestigial discs of workers in different ant species. We compared the wing discs of queens and workers of Myrmica americana, because its life history and development agrees with the assumptions of our mathematical model. In M. americana, there is only one worker caste, and worker larvae possess two pairs of vestigial discs. The disc in Fig. 7B is the vestigial forewing disc in a Myrmica americana worker, and its length along the AP axis was found to be 16.9% of the queen's forewing disc (Fig. 7A). Based on Fig. 6, we predicted that one of the genes in group I (pSmo, pMad, dpp, and Brk) has been interrupted in Myrmica americana. Although the interruption point in the vestigial discs of this species remains to be discovered, the predictions appear consistent with the overall size and shape of the vestigial disc.

DISCUSSION

Our mathematical model of the gene network underlying wing polyphenism in ants is based on *brk* as the key regulatory gene or node that controls growth in wing imaginal discs. We have shown: (1) that in winged ant castes, our model is a good representation of gene expression in the conserved wing patterning network in holometabolous insects; (2) that in wingless ant castes, interruption points unequally affect patterning, target gene expression, and growth; (3) that there are groups of genes that have similar effects on target gene expression and growth; and (4) although genes within these groups occupy different positions in the network, their interruption produce vestigial wing discs that are similar in size and shape. Furthermore, our mathematical model can predict the group of interruption points that have affected growth in vestigial discs in wingless ant castes.

Implications for understanding DSD of the gene network underlying wing polyphenism in ants

Our results hold important implications for understanding how the gene network underlying wing polyphenism in ants evolves in wingless ant castes. It is most likely that a



Fig. 7. Prediction of interruption points in the ant *Myrmica americana* based on size of the wing discs in queen and worker castes. Last larval instar discs of the winged queen (A) and wingless worker (B) castes in the ant *M. americana*. Both images were taken at the same magnification $(20 \times)$, but the scale is different so that the details of the vestigial disc can be seen. The length of the queen (405.43 µM) and worker (68.53 µM) wing discs along the AP axis of the queen disc is measured in µM. The length of the AP axis of the worker disc is approximately 16.9% of the queen disc.

combination of selection and drift has driven the evolution of interruption points in the wing-patterning network in wingless castes. Because interruption points upstream or downstream of *brk* can positively or negatively regulate its expression, any interruption point that maintains upregulation of *brk* will be the main target of stabilizing selection to suppress wing development. Only the genes upstream of the interruption point maintaining upregulation of *brk* will be free to evolve neutrally.

For a new interruption point to evolve in a species, it must assume the role of the previous interruption point by upregulating *brk*, and thus, in suppressing wing development. Simulations based on our model showed that not all genes are equal in their ability to upregulate *brk*. This indicates that not all genes have an equal probability of being interrupted and of evolving into a new interruption point. Furthermore, our simulations also predicted the existence of four groups of genes that have similar effects on growth and target gene expression. Interruption points may evolve neutrally within, but not between these groups. *dpp* and pMad, for example, belong to the same group, and thus, they may neutrally exchange their roles as the interruption point with little functional consequence. In contrast, exchanging an interruption point between genes that belong to different groups, such as *dpp* and Hh, may have serious functional consequence, and thus, could be selected for or against depending on whether or not the exchange is favorable to the fitness of the colony as a whole.

Implications for understanding the dissociation of different levels of biological organization underlying wing polyphenism in ants

Abouheif and Wray (2002) showed that different levels of biological organization underlying wing polyphenism in ants are dissociated. This means that one cannot predict from the level of morphology (no wings) in the worker caste the underlying vestigial disc size or shape, and that one cannot predict from vestigial disc size or shape where the wing-patterning network will be interrupted. For example, the wingless worker castes of *C. lineolata* and *Neoformica nitidiventris*, share similar points of interruption (En), yet their vestigial wing discs differ in size and shape.

An important result that emerged from our model is that there is little correlation between the position of an interruption point in the network and the final size of the wing disc. For example, similar size wing discs can be achieved via the regulation of different genes, such as Hh (10fold downregulation) or Brk (2-fold upregulation). In contrast, different size discs can arise from the same interruption point if it is down- or upregulated to different amounts (i.e., 2-fold vs. 10-fold), as is the case for pSmo. It is therefore possible that the neutral evolution of interruption points within a group may produce similar sized vestigial discs across different species, whereas selection for either an upregulation or downregulation on the same interruption point in different species may produce different sized discs. Both of these scenarios would cause a dissociation between the different levels of biological organization underlying wing polyphenism in ants, i.e. between morphology (no wing), vestigial disc size and shape, and the position of an interruption point in the network.

Implications for understanding the developmental genetic origin of the wing polyphenism in ants

The wing-patterning network was first interrupted somewhere in the ancestral ant lineage leading to all extant ant species. How did wing polyphenism evolve in this ancestral ant lineage? Based on our model and simulations, the first interruption point that evolved may have originated through a slight and gradual, rather than sudden and instantaneous, suppression of wings. Although *brk* is a key regulatory gene, the observation that *brk* suppresses growth in a concentration-dependent manner opens the possibility for a gradual mode of evolution of wing suppression in workers. It may be, for example, that group-II target genes (Hh, *ptc* and Hh_Ptc) were the first genes to be interrupted, as they cause only a modest effect on target gene expression and growth. Interruption points with larger effects on interrupting patterning and growth could then have evolved incrementally, until wings were completely suppressed. Interrupting genes that control patterning and growth along the AP axis in the ancestral ant lineage, set up the conditions for the DSD currently observed in all extant ant species.

Implications for predicting interruption points in the vestigial wing discs in wingless workers

Although our model may have been a useful tool for predicting interruption points in wing discs of workers in Myrmica americana (group I; pSmo, pMad, dpp, Brk), it is only possible to predict the group, rather than a precise interruption point that may be responsible for the relative size of a vestigial disc. One important caveat in attempting to predict the group of interruption with our model is that there are several factors that control the growth or organ size. Truman et al. (2006) showed that growth in the wing imaginal discs of Manduca sexta are controlled by both extrinsic factors, such as hormones and nutrients, and intrinsic factors such as the control of growth via morphogen gradients. Thus, any discrepancies that may arise between empirical data and our predictions may be due to only simulating the intrinsic growth of the imaginal disc. It remains unclear whether brk integrates both extrinsic and intrinsic signals in its control of growth and target gene expression. To test the predictions of our model in the future, one should ideally conduct the following experiments in queens and workers in different ant species: (1) carry out comparative gene expression studies of genes from the predicted group of interruption points; (2) determine wing disc size, shape, and trajectory of growth; and (3) knock-down or over-express the positive or negative regulators of brk from the predicted group (as well as brk itself) to determine the functional consequence of each of the relevant genes.

Conclusions

The patterns of DSD in the gene network underlying wing polyphenism in ants may be a general feature of DSD in plants and animals. The field of developmental and quantitative genetics has revealed the existence of key regulatory genes as well as genes of major effect in almost every developmental process (Haag and True 2001). Furthermore, the structure of gene networks is such that key genes are

commonly embedded in complex, nonlinear interactions, with upstream regulators and downstream targets. Thus, a combination of selection and drift may generally drive DSD in gene networks that underlie homologous phenotypes. This prediction can be tested as more comparative data becomes available in a wide range of taxa. Finally, the mathematical approach we presented in this article can be generally applied to study the reduction or modification of wings in holometabolous insects, such as the reduction of hindwings in *Drosophila* (Weatherbee et al. 1998; Crickmore and Mann 2006), wings in female bagworm moths (Sattler 1991), and wings in some male fig wasps (Cook et al. 1997).

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SUPPLEMENTARY MATERIALS

The following material is available for this article online:

Appendix S1. Initial and boundary conditions for the model.

Appendix S2. Estimation of default parameters used in the numerical simulations.

Table S1. Estimation of parameters from the literature for Equations (1-15).

Fig. S1. Initial conditions used in simulations. Initial conditions for equations (1)–(15) used in all the simulations. Initial profiles for En (a), *ptc* (b), and Ptc (c) correspond to conditions (I. C. 1–3). All other genes are assumed to be initially zero (I. C. 4).

Fig. S2. Simulated gene expression profiles. Steadystate simulated profiles for Hh (a), *ptc* (b), Ptc (c), pSmo (d), Dpp (e), *tkv* (f), *omb* (g), and Dad (h). The predicted expression profiles for these genes showed a good qualitative fit to their observed expression profiles in *Drosophila* (data not shown).

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