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Reflections on Bateson's rule: Solving an old riddle about why extra legs are mirror-symmetric

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Abstract

William Bateson was an obsessive observer of animal oddities, and at some point in his herculean survey of museum collections leading up to his monumental 1894 monograph (*Materials for the study of variation*), he noticed a peculiar trend among the preserved specimens (mainly insects) that possessed extra legs: multiple legs that branched from the same socket tended to be mirror images of their adjacent neighbors. He did not know why. These symmetry relationships have come to be known as Bateson's rule, and they have defied a satisfactory explanation for 125 years. In the past few decades, tantalizing clues have emerged from various lines of investigation, and those lines have converged on a possible solution. An attempt is made here to fit all of those clues together to form a coherent picture of the etiology. Two case studies have proven to be pivotal: a fly mutant whose extra legs are caused by patches of dying cells and a frog syndrome whose extra legs are caused by a parasitic flatworm. The conclusion reached is that the extra legs of insects and vertebrates obey Bateson's rule for the same reason, but that reason has nothing to do with the specific molecules in their signaling pathways. Rather, it is an emergent property of the circuitry of the pathways and their polarized alignments along the limb axes. A parade of theoretical models have tried and failed to crack this mystery in the past, and they are reviewed here as part of the narrative.

KEYWORDS
Bateson's rule, emergent property, genetic circuitry, limb regeneration, symmetry

1 | INTRODUCTION

William Bateson (1861–1926) is famous for having coined the term genetics, but to aficionados of evolution and development, he is also revered for his 1894 opus *Materials for the study of variation treated with especial regard to discontinuity in the origin of species* (Bateson, 1894). That classic is a gargantuan Victorian monograph, arguably on a par with Darwin's *Origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.*

Indeed, Bateson had Darwin on his mind when he wrote the book. For Bateson the lingering question that Darwin left unanswered was the extent to which species arise via the gradual accumulation of miniscule anatomical changes over long periods of time. The alternative that intrigued Bateson was that evolution might proceed primarily by sudden jumps—what he called "discontinuities"—that arise as dramatic departures from the norm (Gillham, 2001).

His book cataloged hundreds of such deviations—both animal and plant. P. T. Barnum would have called them freaks. Richard Goldschmidt called them "hopeful monsters" (Piternick, 1980) to stress their potential as progenitors of new species, assuming they confer an adaptive benefit (Diogo, Guinard, & Diaz, 2017). Saltations of this sort may have founded a few taxa (Lebreton et al., 2018), but they surely did not play the kind of cardinal role that Bateson and Goldschmidt envisioned (Dietrich, 2003; Theissen, 2006).

Buried deep in Bateson's tome is a fascinating illustration (Figure 1b). It is a drawing of a wooden contraption he built, with carved
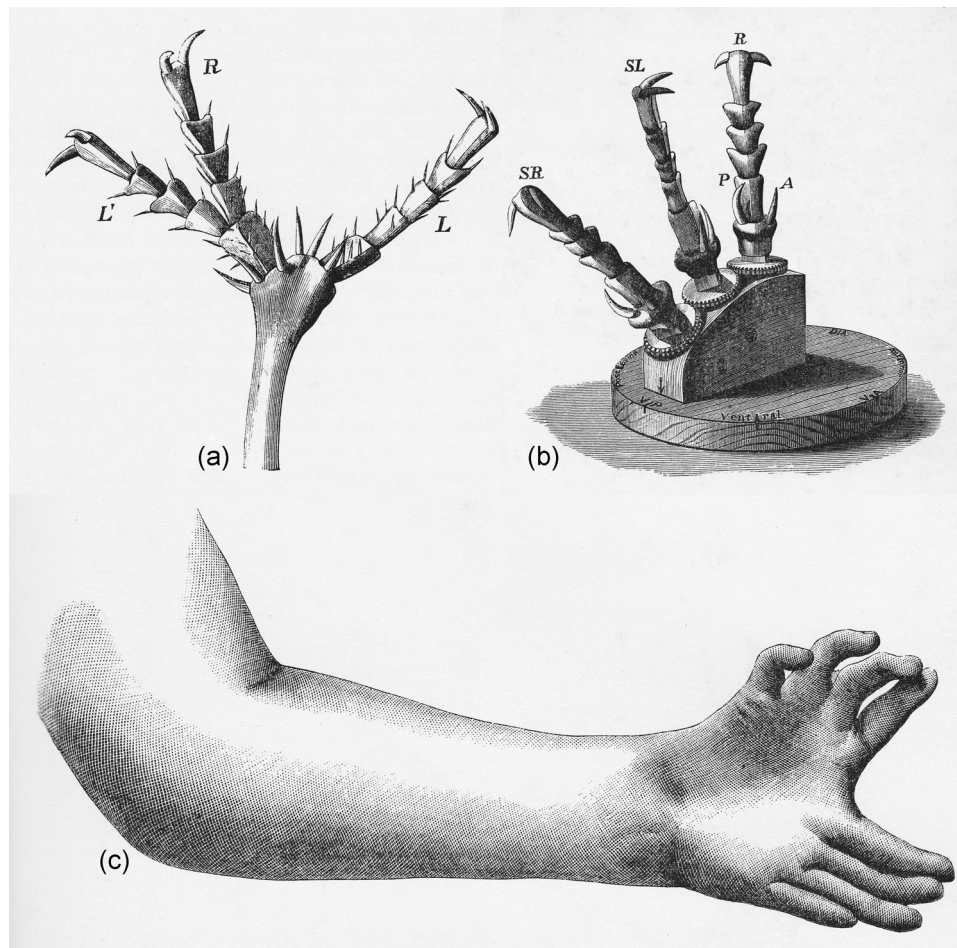
FIGURE 1 Drawings of a triplication (a), a duplication (c), and a contraption that simulates triplications (b) from Bateson's (1894) monograph (Bateson, 1894). (a) Distal portion of the left middle leg of a ground beetle (family Carabidae) with three tarsi emanating from a single tibia (Case #742): the normal tarsus (L), an extra right tarsus (R), and an extra left tarsus (L'). (b) Wooden device showing the relations between "supernumerary" legs (SR and SL) and the normal leg (R), with A and P marking anterior and posterior tibial spurs (his fig. 153). (c) Double-hand anomaly (ventral aspect) in the left arm of a woman (Case #495), whose right arm was normal—a phenotype that vexed Bateson because a right hand on the left side of the body represents the emergence of a fundamental feature of animals (bilateral symmetry) in a context (unilateral) where it does not belong (Bateson, 1971). This may be an instance of ulnar dimelia syndrome, and feet can be doubled too (Brower, Wootton-Gorges, Costouros, Boakes, & Greenspan, 2003; Hatchwell & Dennis, 1996; Klaassen, Shoja, Tubbs, & Loukas, 2011). Bateson cited a similar anomaly in a man (Case #492) who considered his condition to be an asset for his job (machinist) and especially for playing the piano. Duplications like the one in (c) are not rare (70 cases reported as of 2015 (Rabah, Salati, & Wani, 2008; Tomaszewski & Bulandra, 2015)), but limb triplications are virtually nonexistent in amniotes, unlike the situation in frogs, where they are common (see text). This disparity may have to do in part with how different taxa deploy signaling molecules in their limb buds. To wit, frogs appear to use Fgf8 along their A-P axis, while birds and mammals employ it along the proximal-distal axis (Delgado & Torres, 2017). The exact location of the Fgf8 zone in nonmodel species is less clear. Another unresolved mystery is why angles between mirror planes in triplicated limbs vary so widely (cf. device in (b)). The present essay mainly focuses on cases where the planes are perpendicular to the A-P axis in frogs and the D-V axis in flies. A-P, anterior-posterior; D-V, dorsal-ventral; Fgf8, fibroblast growth factor-8

wax renditions of three oversize insect legs sprouting from it like an upside-down beetle trying to right itself. The legs are geared together so that when one rotates, the others do also, but the flanking legs spin counter to the center one like ballerinas in a mirrored studio.

Bateson's device demonstrated the range of symmetries he observed in the branched appendages of insects, crustaceans, and other animals that comprised a distinct category of deformity in his hefty catalog. He focused mainly on triplicated limbs (e.g., Figure 1a) and distilled two recurring themes from such specimens:

I. The long axes of the normal appendage and of the two extra appendages are in one plane: of the two extra appendages one is therefore nearer to the axis of the normal appendage and the other is remoter from it.

II. The nearer of the two extra appendages is in structure and position formed as the image of the normal appendage in a plane mirror placed between the normal appendage and the nearer one; and the remoter appendage is the image of the nearer in a plane mirror similarly placed between the two extra appendages.

These relationships among extra legs have come to be called Bateson's rule (Bateson, 1971), though they should technically be called *rules* (plural) since there are two axioms. Why these trends exist is unknown. They have remained a beguiling mystery for 125 years, as has the etiology of the branched legs themselves.

Mounting evidence for "deep homology" (Held, 2017)—a shared legacy of genetic circuitry for appendages and other structures— suggests that animals might obey Bateson's rule because they construct their limbs via the same ancient genetic toolkit, but the line of reasoning pursued here leads to a starkly different conclusion: diverse taxa obey Bateson's rule because more than one limb cannot emerge from the same socket without positional signals leaking across the "limb/limb" interface. This "bleeding" of those signals launches an intervening limb of backward polarity ("bmil"), culminating in a palindromic "limb/bmil/limb" Batesonian sequence.

2 | PIONEERING MODELS

Wolpert (1996) revolutionized the field of developmental biology with his concept of positional information. He introduced this new way of thinking in 1968 by posing what he called the French flag problem (Wolpert, 1968), and a year later he detailed his solution for that problem in the *Journal of Theoretical Biology* (Wolpert, 1969). His basic idea was that cells know what to do based on where they are. To wit, cells adopt states of differentiation (bone, muscle, nerve, etc.) based on their locations within Cartesian coordinate systems. The (x, y) coordinates of cells in two-dimensional epithelial sheets are encoded as distances from fixed axes. Distances were thought to be specified by chemicals (morphogens) that (a) are secreted by the source axes, (b) diffuse randomly, and (c) decrease in concentration with increasing distance to form linear gradients. Wolpert's gradient model ruled as a paradigm, virtually unchallenged until 1976.

In 1976 the "Polar Coordinate Model" debuted in *Science* magazine (French, Bryant, & Bryant, 1976). Polar coordinates are familiar as the latitude and longitude lines that designate locations on the earth. A growing limb would correspond to the northern hemisphere, with its future tip being the north pole and its base's perimeter being the equator. Looking down on the north pole, longitude lines resemble the hours on a clockface, and latitude lines are concentric circles. Similarly, a cell's coordinates inside a limb rudiment (r, θ) would be its radial distance from the center of the clock (latitude), and its hour-hand angle (longitude) from the 12 o'clock meridian.

This model was the brainchild of Vernon French, Peter Bryant, and Susan Bryant, all of whom were studying limb regeneration, albeit in different animals: cockroaches (French), flies (P. Bryant), and salamanders (S. Bryant), respectively. Their model explained a wide variety of experimental results from all three taxa. The outcome most relevant to Bateson's rule was the triplication, which could be elicited in cockroaches and salamanders by grafting the tip (or blastema) of a left leg onto the stump of a right leg, or vice versa (Figure 2).

In each such case, the transplant and stump clockfaces would be reversed relative to one another along one axis, and a "supernumerary" clockface would emerge at each terminus due to confrontation of coordinates from opposite sides of the clock. Each new clockface would produce a mirror-image leg via intercalary growth that bridges the gaps between those coordinates, as per the model's rules:

I. Shortest intercalation rule: When normally nonadjacent positional values in either the circular or the radial sequence are confronted in a graft combination or as a result of wound healing, growth occurs at the junction until cells with all the intermediate positional values have been intercalated; then growth ceases. The circular sequence is continuous and the position 12/0 does not imply a boundary having unique properties. This continuity of the circular sequence means that there are two possible sets of intermediate values between any two nonadjacent positional values. For example, juxtaposition of cells with values 3 and 6 gives two possible sequences of intermediate values: 3 (4, 5) 6 and 3 (2, 1, 12/0, 11, 10, 9, 8, 7) 6. A critical stipulation of the model is that when cells with nonadjacent positional values in the circular sequence are brought into contact, intercalation is always by the shorter of the two possible routes.

II. Complete circle rule for distal transformation: The entire circular sequence at a particular radial level may undergo distal transformation to produce cells with all of the more central (distal) positional values. We propose that this distal transformation occurs only when cells with a complete circular sequence of positional values are either exposed at an amputation site or generated by intercalation.

These rules were predicated on the assumption that adjacent cells can sense one another's coordinates and respond accordingly (by intercalary growth). Hence, the polar coordinate model differed from Wolpert's gradient model in its obligate reliance on direct cell-surface contacts instead of diffusible morphogens, though morphogens could theoretically be assigning polar coordinates during development.

The complete circle rule was later abandoned when artificially constructed, symmetric "double-half" limbs were shown to be capable of outgrowth, despite having fewer than half of the angular values (Bryant, French, & Bryant, 1981). In its place, the authors proposed a "distalization" rule that was just an extension of the shortest intercalation rule, amended to compel new cells to adopt distal identities, as emphasized by the final (italicized) sentence.

II. Distalization rule: To achieve distal outgrowth the new cells generated during circumferential intercalation at the growing tip of the appendage must adopt positional values that are more distal than those of the pre-existing cells at the wound edge. We propose that this comes about as a result of a strictly local interaction as follows: during intercalation, a newly generated cell will normally adopt a positional value which is intermediate between those of the confronted cells. However, *if this represents a positional value that is identical to that of a preexisting adjacent cell...then the new cell is instead forced to adopt a positional value that is more distal than that of the preexisting cell.*
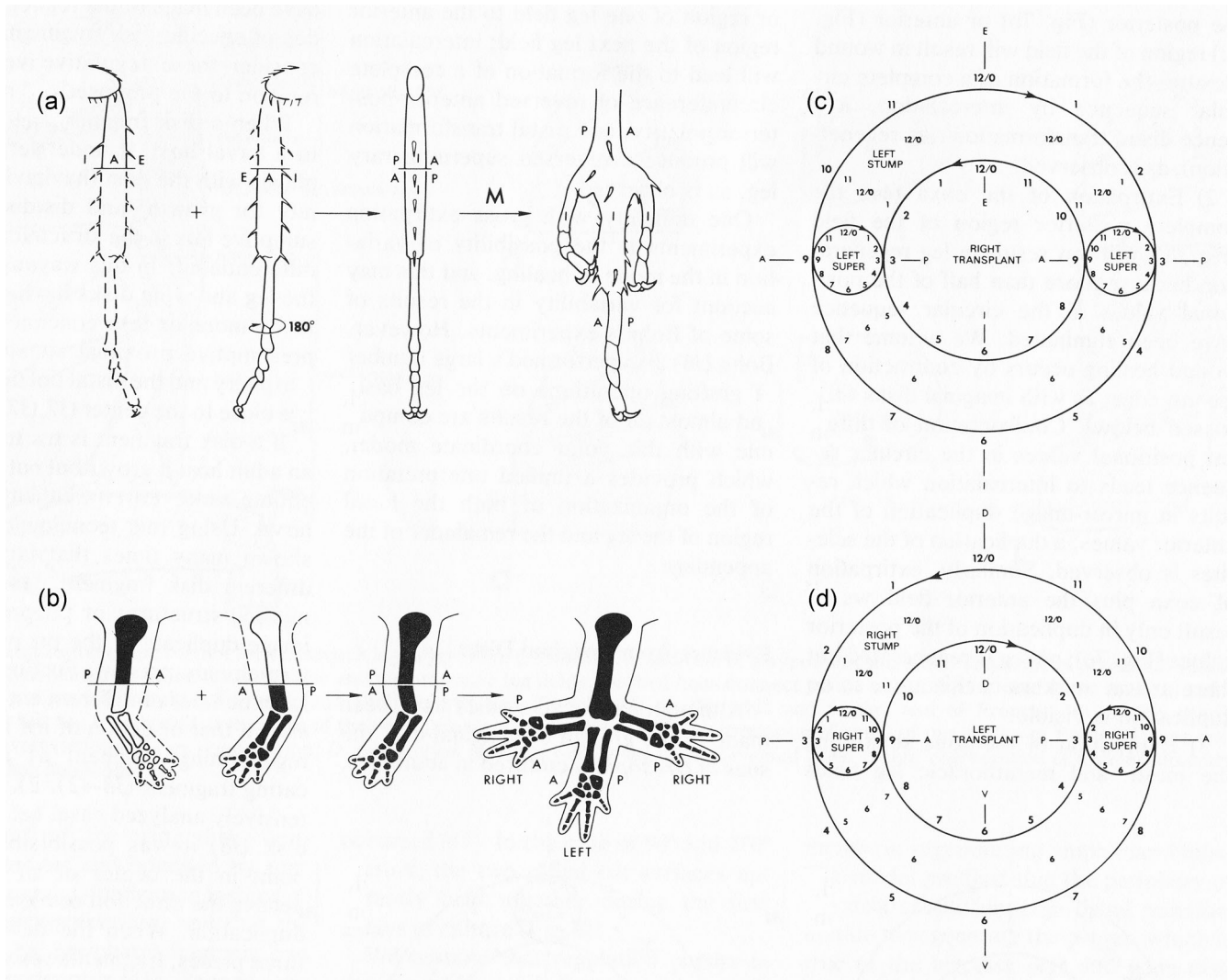
FIGURE 2 Polar coordinate model's explanation for why two new limbs (supers) grow out when a limb (a) or blastema (b) from one side of the body is grafted onto a contralateral amputation stump. In each case, the anterior-posterior axes of transplant (inner circle) and stump (outer circle) are reversed, forcing cells with opposite values (3 vs. 9) to meet (c,d). These disparities provoke intercalation by the shorter route (new clockfaces), creating two new limbs that obey Bateson's rule. (a,c) Cockroach. (b,d) Newt. Used with permission from *Science/RightsLink* (French et al., 1976)

3 | ENTER THE EXTRA-LEGGED FLY

The legs that Bateson analyzed had arisen spontaneously, rather than being induced surgically, so the explanations sketched in Figure 2 did little to solve the riddle. However, a strain of the fruit fly *Drosophila melanogaster* was being studied around this time that seemed fortuitously custom-made for probing Bateson's rule, like a deus-ex-machina that drops into the play at just the right time to save the day.

The strain carried the mutation *lethal(1)ts726*, which, as its name suggests, kills flies before they hatch from the pupal case (*lethal*) and exhibits temperature-sensitivity (*ts*): homozygotes look normal if they are raised at 22°C, but they tend to develop extra, mirror-image legs if they are exposed to 29°C during the larval period (Russell, 1974; Russell, Girton, & Morgan, 1977). Heat treatments in the early part of that period cause *duplications* (Girton & Russell, 1980),
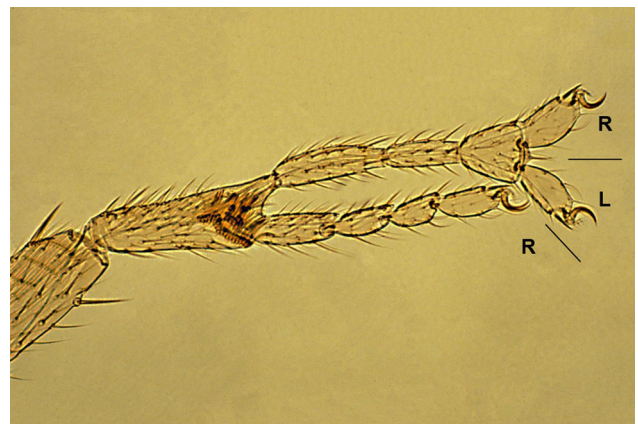

FIGURE 3 Triplicated right foreleg (tarsal region only) from a *lethal(1)ts726* fly kept at 29°C for two days in the third instar. R, right branch and L, left branch. The R/L/R pattern conforms with Bateson's rule. Photo courtesy of Jack Girton

FIGURE 4 Development of a second-leg disc. Black outlines (a–d) are sagittal sections (see timeline). Asterisk marks the future claws. Cell boundaries are omitted. (a) Mid-2nd instar, when the disc acquires a lumen (Auerbach, 1936; Mandaravally Madhavan & Schneiderman, 1977; McKay, Estella, & Mann, 2009). Cells remain cuboidal until 3rd instar, when one side thickens and the other flattens; both remain monolayers. (b) Late-3rd instar. The thick side has concentric folds (top view in (f); Condic, Fristrom, & Fristrom, 1991). (c,d) Early pupal period. Folds telescope out, and the leg everts through the stalk (Pastor-Pareja, Grawe, Martín-Blanco, & García-Bellido, 2004) to form a hollow cylinder that secretes the adult cuticle. (e) Adult leg (anterior view). Adapted from (Held, 2002)
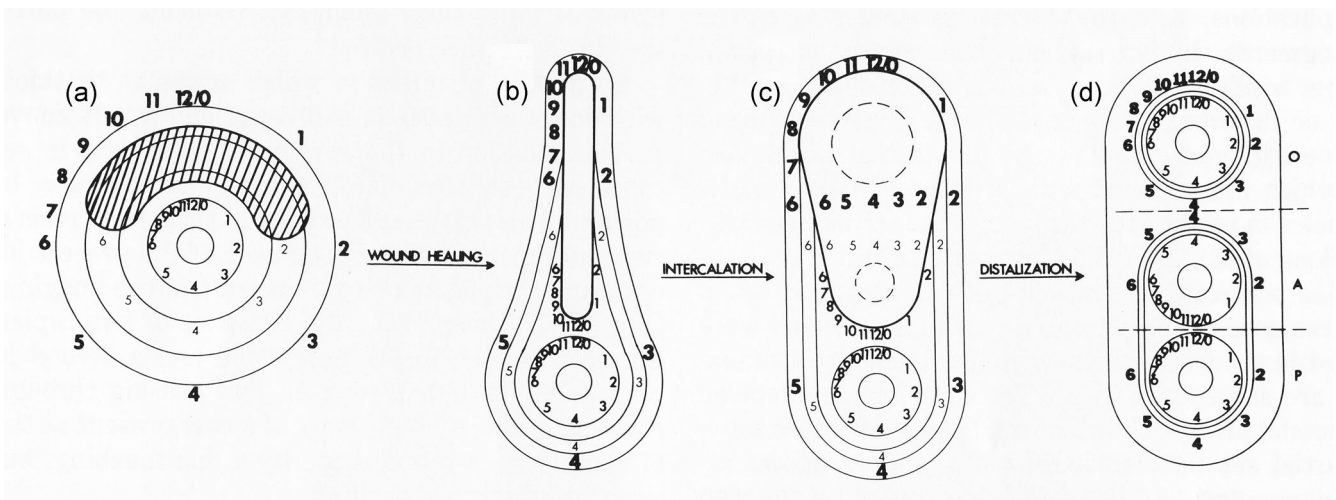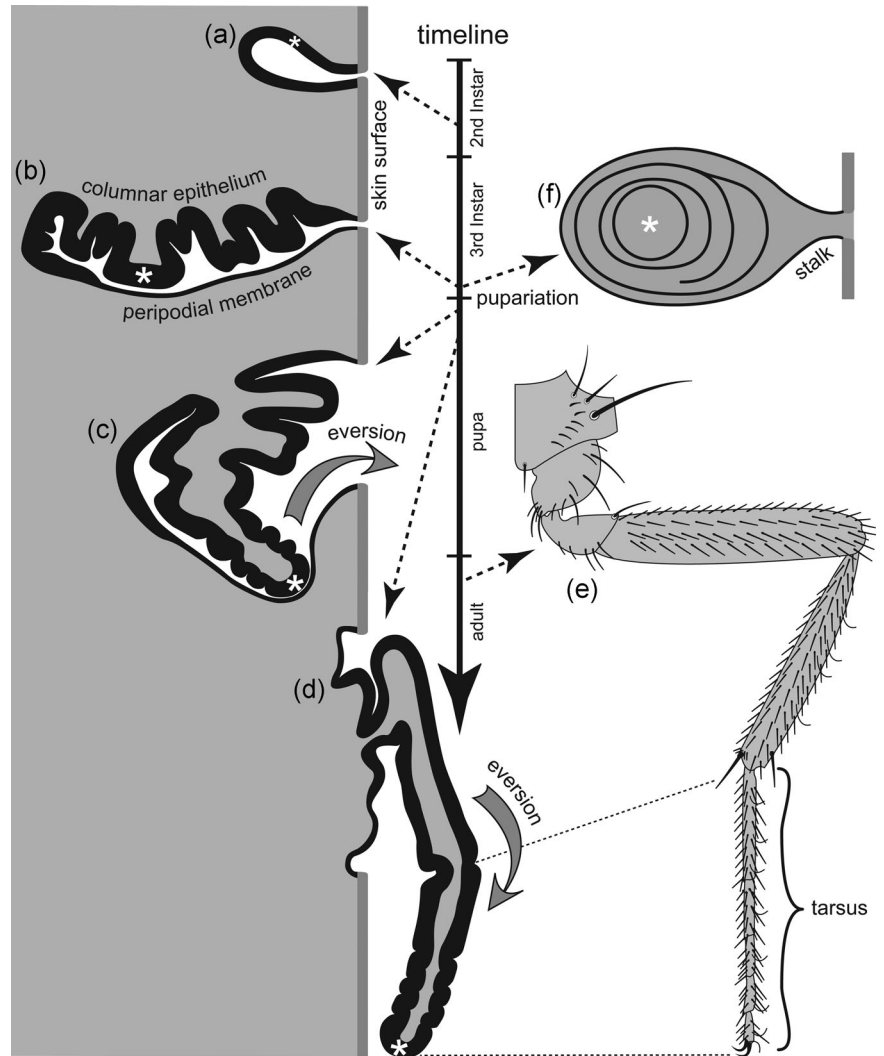

FIGURE 5 Polar coordinate model's explanation for why two new legs grow out from a *lethal(1)ts726* leg disc when a sausage-shaped area (shaded) undergoes apoptosis (a). The wound rips open during telescoping (b) and gets stretched in a perpendicular (vertical) direction, confronting cells from opposite sides. Those confrontations lead to intercalation (numbers inside oval) by the shorter route (c) and ultimately to two new clockfaces (d), each of which forms an extra leg branch. Used with permission from *Developmental Biology* (RightsLink)
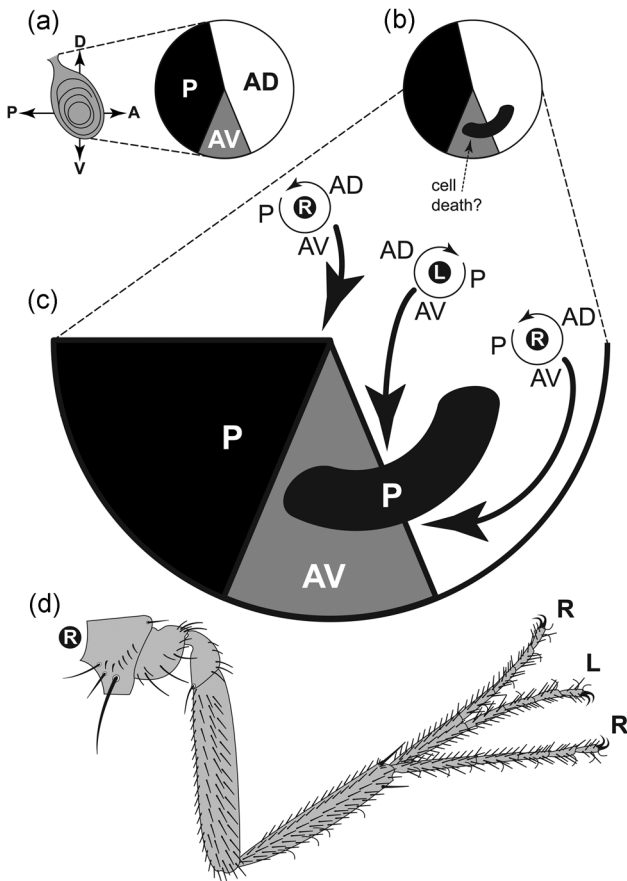
FIGURE 6 Boundary model's explanation for why two new legs grow out from a *lethal(1)ts726* leg disc when a sausage-shaped area (shaded) undergoes apoptosis. (a) Leg disc (A, anterior; P, posterior; D, dorsal; and V, ventral), and a simplified schematic (circle) showing P (black) and A (=AD + AV) cell-lineage compartments. Meinhardt thought the A region might contain two subdomains, AD (white) and AV (gray), so that the intersection point of P with AD and AV triggers leg outgrowth. (b) Idealized cartoon of a *lethal(1)ts726* leg disc that is suffering a patch of cell death (black arc). (c) Enlarged region of the lower half of the disc, wherein the dying cells have switched their state from A to P, causing two new AD/AV/P intersection points to arise flanking the dying swath. An extra leg will grow out from each such point, whose handedness is set by the "P→AV→AD→P" sequence around the circle. (d) The resulting triplication (which started out as a right leg) obeys Bateson's rule

whereas heat treatments in the latter half cause *triplications* (Girton, 1981) (Figure 3)—precisely the kinds of phenotypes that impelled Bateson to formulate his rule in the first place.

The branched-leg phenotypes of *lethal(1)ts726* were ultimately traced to specific regions of apoptosis (i.e., programmed cell death (Monier & Suzanne, 2015)), as explained below (Girton & Kumor, 1985). To comprehend this etiology, however, it is first necessary to conceptualize the geometry of how normal fly legs develop.

Fly legs grow inside the larval body as hollow sacs called imaginal discs (Held, 2002), and each leg disc has concentric folds that telescope out during metamorphosis to form segments of the adult leg (Figure 4). Surgical experiments on leg discs had been cited as evidence for the polar coordinate model, but the specific quadrant of

the foreleg disc that regenerates (vs. duplicates) was later shown to only be capable of doing so because of an unsuspected region of cells beyond the main epithelium (Gibson & Schubiger, 1999), so the inferred pluripotency of that quadrant has had to be reinterpreted very differently (Gibson & Schubiger, 2000).

The *lethal(1)ts726* mutation does not kill cells randomly. Rather, it causes apoptosis in swaths that curve around the disc in arcs, and the extent of apoptosis is correlated with the frequency of leg duplication and triplication (Girton & Kumor, 1985). Proof of a *causal* relationship (i.e., cell death causing leg branching) was obtained by a clever approach. Leg discs were removed from wild-type (nonmutant) larvae, placed on a slide, and exposed to a laser beam that was raked across the disc to kill cells in the same general sausage-shaped swath where cells die in *lethal(1)ts726* mutant discs. When these lasered discs were allowed to metamorphose, the resulting adult legs displayed duplications and triplications (Girton & Berns, 1982). The laser had "phenocopied" the mutant phenotype (Landauer, 1959).

In 1981 the triplications of *lethal(1)ts726* were interpreted in terms of the polar coordinate model (Bryant et al., 1981; Girton, 1981) by assuming that the swath of dying cells gapes open, with the subsequent telescoping of the disc causing that hole to heal incorrectly—as if a person were to suffer lockjaw while yawning so that his lips could only close laterally, rather than vertically as they should (Figure 5).

There are several difficulties with this scenario. First, the drastic contortions that are needed for it to work are unlikely to occur in the laser-ablated discs that metamorphosed as implants and hence did not undergo normal morphogenesis, yet nevertheless produced triplications. Second, the presumed clustering of more than half of the angular values in the upper left (medial) quadrant (6–12/0), which is also critical here, was invalidated by subsequent experiments (Gibson & Schubiger, 1999). Finally, there is no evidence that the dying cells in the sausage-shaped area are cleared by macrophages quickly enough for the cells around the perimeter to ever come into contact (Jack Girton, personal communication, June 22, 2019)—precluding the cell-to-cell contact that is a sine qua non of the polar coordinate model (Bryant et al., 1981) and a mandatory prerequisite for intercalation leading to triplication.

4 | THE BOUNDARY MODEL

In 1983 Hans Meinhardt proposed a clever alternative explanation for how *lethal(1)ts726* causes triplications, and his "Boundary Model" also accounted for Bateson's rule (Meinhardt, 1983). He began by noting that the leg disc is organized into anterior (A) and posterior (P) cell-lineage compartments, and that the A/P line always runs through the center of the disc. He reasoned that this point (the future tip of the leg) could be uniquely specified if there were a comparable boundary along the dorsal-ventral (D-V) axis, though no such lineage constraint actually exists. Nevertheless, he asserted (ad hoc) that the A compartment must be subdivided into some sort of dorsal (AD) and ventral (AV) domains (Figure 6a), and that distal outgrowth will
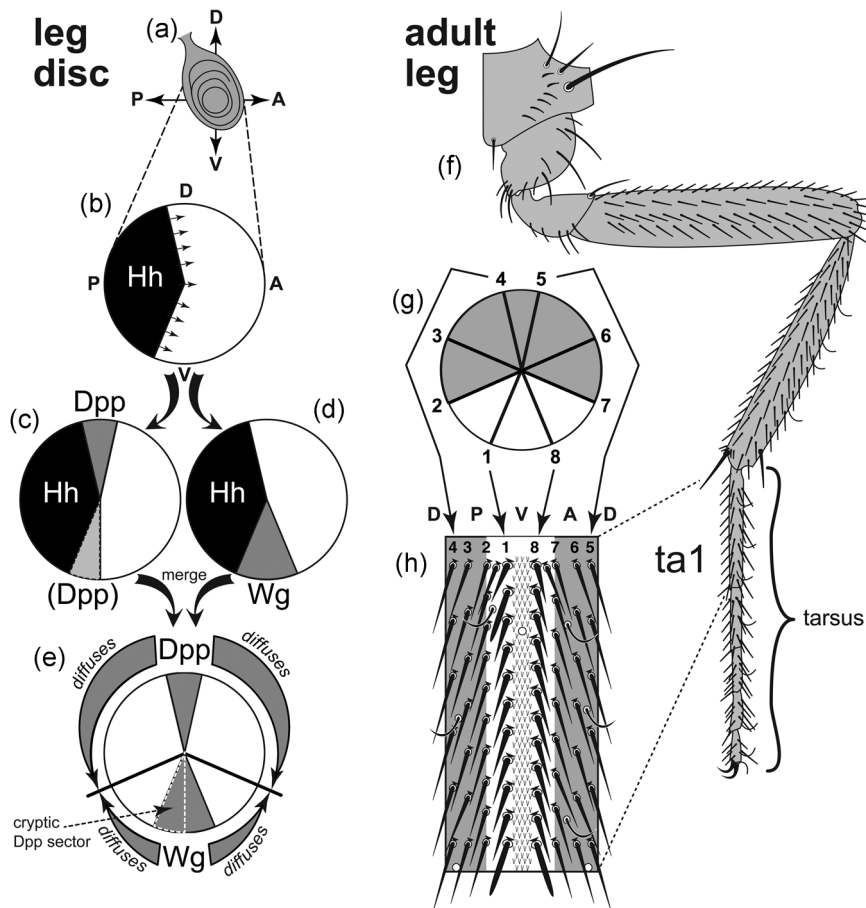
FIGURE 7 Morphogen control of fly leg development. (a) Left second-leg disc (cf. Figure 4). Directions: dorsal (D), ventral (V), anterior (A), and posterior (P). (b) Schematic of the disc as a circle. As in the boundary model (Figure 6), the P compartment (black) is critical, but in a different way. It expresses Hh, which diffuses (arrows) across the A/P boundary to activate Dpp and Wg. (c) Dpp is expressed strongly in a slim D sector and weakly (dotted outline) in a slim V sector (Theisen et al., 2007). (d) Wg is expressed in a wide V sector (Baker, 2011). (e) Complementary "spheres of influence" (diffusion ranges?) for Dpp (~225°) and Wg (~135°) are marked by thick lines (Held & Heup, 1996). Dpp's V sector (dashed outline) is inert ("cryptic"). (f) Left second leg of an adult, anterior view. The tarsus has five segments, the most proximal of which is ta1. (g) Fate map of tarsal bristle rows in the second-leg disc (Held, 1979). This pinwheel becomes a parallel array (h) by telescoping of the disc during metamorphosis (Figure 4) (Campbell & Tomlinson, 1995). The realms ruled by Dpp and Wg are shaded gray and white, respectively, in (g) and (h). (h) Panoramic map of the bristle pattern, drawn as if ta1 had been cut along its D midline and flattened to show its whole surface. Dpp, decapentaplegic; Hh, hedgehog; Wg, wingless

always occur wherever this imaginary AD/AV boundary intersects the bona fide A/P boundary (Meinhardt, 2009).

Evidence was then beginning to accumulate that apoptosis can cause cells to change their identity before they expire (Szabad, Simpson, & Nöthiger, 1979), and the case has grown stronger since then (Worley, Alexander, & Hariharan, 2018), so Meinhardt simply connected all of these dots: (a) the mutation causes curved zones of cell death (Figure 6b), (b) the cells in such an A zone could switch identity from A to P, in which case (c) a thick arc of P cells might now overlap the AD/AV boundary (Figure 6c), and if so, then (d) two new AD/AV/P intersection points would ensue, and (e) two new legs would branch out (Figure 6d). He explained that the legs are mirror symmetric because the clockwise or counterclockwise (P→AV→AD) order of the areas around the intersection point would dictate each leg's handedness.

Despite the elegance of Meinhardt's model, it lacked enough supporting evidence to be widely accepted at the time (Marsh &

Theisen, 1999). Nevertheless, its fortunes were about to change. In 1988 researchers showed that a powerful morphogen—wingless (Wg)—was being expressed at high levels in Meinhardt's imaginary AV sector (Baker, 1988a, 1988b), and this discovery buttressed the model's validity. Indeed, researchers who were trying to decipher Wg's role relied on the boundary model to devise their own testable hypotheses (Baker, 2011), and some key tenets of its overall scenario have endured to this day, albeit in altered form as recounted below (Marsh & Theisen, 1999).

5 | THE DECAPENTAPLEGIC (DPP)-WG MODEL

In 1990, a second morphogen was mapped along the D-V axis, but it did not match the third domain of Meinhardt's triumvirate (Masucci,
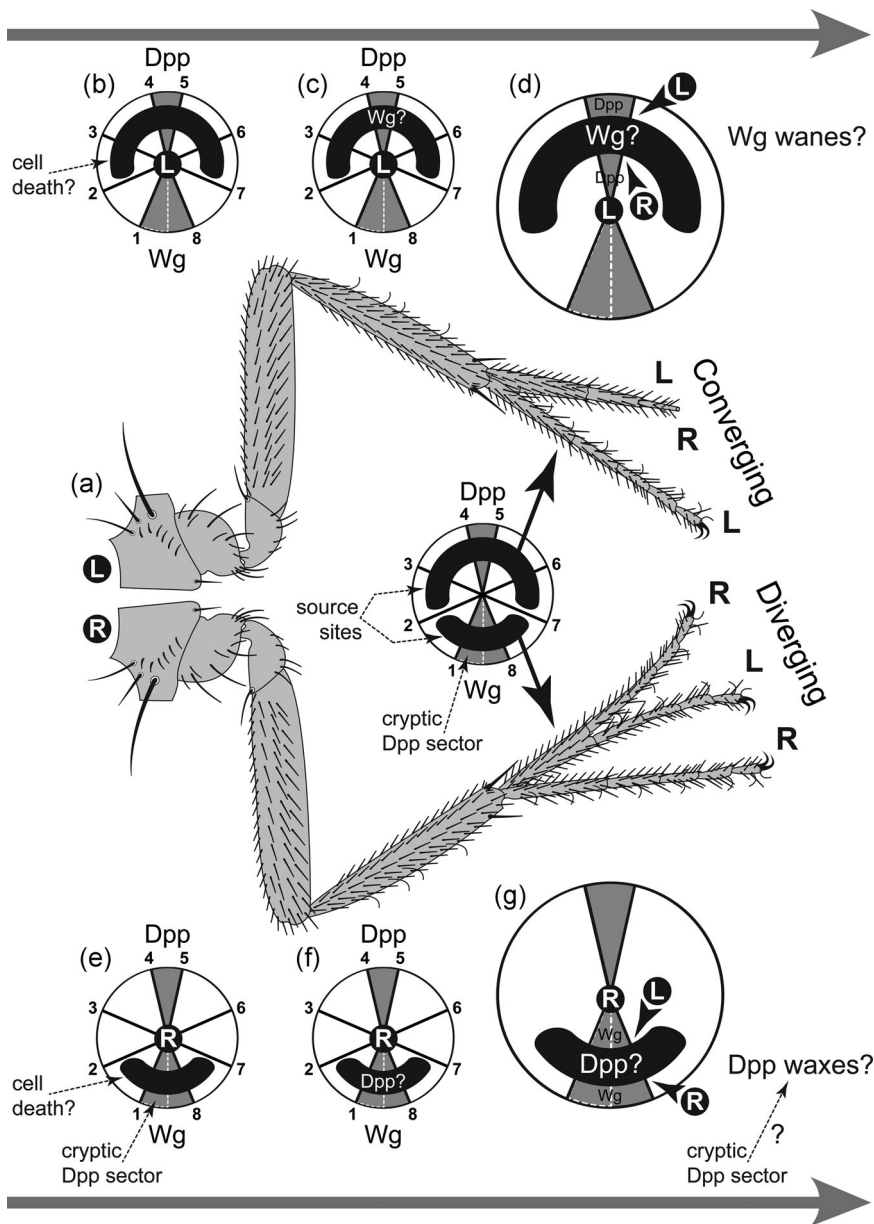
FIGURE 8 How converging versus diverging triplications might arise in *lethal (1)ts726* leg discs. Both types obey Bateson's rule. (a) Converging (above) and diverging (below) triplications emerge from different sausage-shaped areas (black). Gray sectors are expression domains of Dpp and Wg, and pinwheel spokes are future bristle rows (1–8; cf. Figure 7). Converging outgrowths taper distally and come from a dorsal arc, while diverging ones branch distally and come from a ventral one. (b–d) Converging etiology, starting with a left (L) leg. Cell death is presumed to occur in the black area (b) and to elicit Wg expression (c), causing two new points of Dpp-Wg contact and hence two new legs (d). The upper leg retains its handedness (L), while the other one flips (R, right). The fused legs taper, possibly because the ectopic Wg wanes. (e–g) Diverging etiology, starting with a right (R) leg. Cell death is presumed to occur in the black area (e) and to elicit Wg expression (f), causing two new points of Dpp-Wg contact and hence two new legs (g). One keeps its handedness (R), while the other flips (L). Each branch gets more complete circumferentially as it grows, possibly due to rising levels of Dpp. Dpp, decapentaplegic; Wg, wingless

Miltenberger, & Hoffmann, 1990). The morphogen is called Dpp after the 15 appendicular defects that arise when the gene is disabled. The Dpp sector was thinner than the opposite Wg sector, and there was a minor sector of reduced Dpp expression in the heart of the Wg zone. Dpp's V sector was later shown to be nonfunctional (Held & Heup, 1996), so only Dpp's D sector is relevant to the patterning process. The overall mechanism was deciphered in the ensuing few years, and it is summarized in Figure 7.

Dpp and Wg serve as D and V morphogens, respectively, and each of them has its own autostimulatory positive feedback loop (Campbell, 2002; Marsh & Theisen, 1999). They inhibit one another (Ayala-Carmago, Ekas, Flaherty, Baeg, & Bach, 2007; Kojima, 2004) but interact synergistically to trigger outgrowth where high levels of their expression merge in the center of the disc (Campbell & Tomlinson, 1995; Campbell, Weaver, & Tomlinson, 1993). This "Dpp + Wg" ignition "app" was proven in 1993 when clones of

Wg-expressing cells were randomly initiated (Struhl & Basler, 1993). The clones caused triplications, but only when they hit the Dpp sector. Interaction between Dpp and Wg is necessary and sufficient for outgrowth, and this fact formed the basis for the Dpp-Wg Model proposed by Gerard Campbell and Andrew Tomlinson in 1993 (Campbell et al., 1993) and refined in 1995 (Campbell & Tomlinson, 1995).

6 | CROSS-EXAMINING THE EXTRA-LEGGED FLY

The most tantalizing clue that *lethal(1)ts726* offers for solving the riddle of Bateson's rule is that its triplications come in two types: "converging" ones taper, while "diverging" ones split into branches that get progressively more complete (Figure 8). The most baffling

FIGURE 9 How duplications might arise in *lethal(1)ts726* leg discs. (a) Left (L) second leg, which has sprouted a mirror-image right (R) duplicate (the normal R partner is not shown). The duplicate comes from the sausage-shaped area (black) (Girton & Russell, 1980; Russell et al., 1977). Gray sectors are expression domains of Dpp and Wg, and pinwheel spokes are future bristle rows (1–8; cf. Figure 7). (b–d) Proposed sequence of events. Cell death is presumed to occur in the black area (b) and to elicit Dpp expression (c), causing one new point of Dpp-Wg contact and hence one new leg (d), which is left-handed due to the inverted polarity of its dorsal-ventral axis. Dpp, decapentaplegic; Wg, wingless
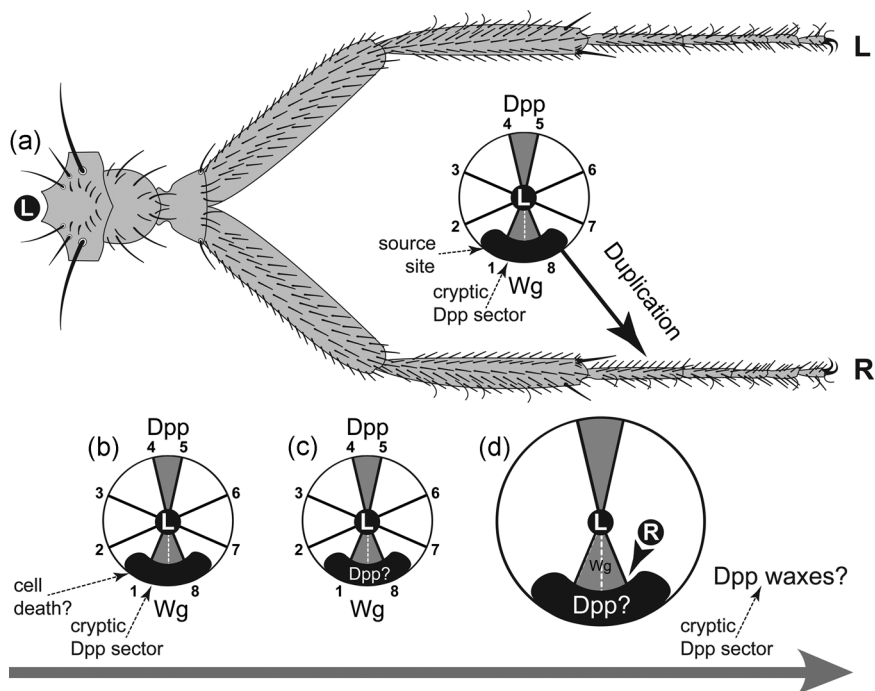
aspect of this dichotomy is that converging outgrowths stem from the D side of the disc, while "diverging" outgrowths emerge from the V side. Why should this be?

If cell death is occurring in the same areas that sprout extra legs (Figure 8b,e), then those legs can be explained by assuming that apoptosis activates Wg in the Dpp sector of some discs (Figure 8c) and Dpp in the Wg sector of others (Figure 8f). The ensuing proximity of Dpp- and Wg-expressing cells on either side of the death zone should then trigger a new leg to arise (Figure 8d,g; Campbell & Tomlinson, 1995), and the neighboring members of each such pair will have opposite handedness in conformity with Bateson's rule (Figure 8a). Indeed, it turns out that Wg and Dpp are both spurred to high levels of expression when tissue is ablated by X-irradiation (Pérez-Garijo, Shlevkov, & Morata, 2009) or by tweaking genes of the apoptotic pathway (Hariharan & Serras, 2017; Smith-Bolton, Worley, Kanda, & Hariharan, 2009), and regeneration can be stopped abruptly in its tracks by blocking Wg (Harris, Setiawan, Saul, & Hariharan, 2016).

Because Wg and Dpp antagonize one another so fiercely (Jiang & Struhl, 1996; Marsh & Theisen, 1999), it is hard to see how they could emerge in the heart of "enemy territory" without being snuffed out quickly. The solution to this paradox appears to be that apoptosis induces amnesia before it delivers the coup de grace: it causes cells to "forget" who they are by erasing memory markers (polycomb proteins, etc.) from their identity genes (Worley et al., 2018). Thus, a cluster of cells in the Dpp sector, say, could forget its Dpp identity and "re-boot" in either its old Dpp state or a new Wg one (Morata, Shlevkov, & Pérez-Garijo, 2011). Patches that return to a Dpp state would blend back into the background, yielding a normal leg after healing, while those that reawaken in a Wg state would elicit extra legs. If the choice between these options were random, then no more

than 50% of legs in the *lethal(1)ts726* flies should ever be triplex. Indeed, the actual maximum is 36% (Girton, 1981).

So far so good, but what enables triplex legs that arise ventrally to diverge? Their secret "superpower" may be their cryptic Dpp sector. Dpp is expressed (albeit at a low level) in the Wg sector (Masucci et al., 1990), so it could easily be launched from that platform to an intensity that could sustain outgrowth. There is no comparable sector of Wg in the Dpp area (though Wg can manage to bloom when Dpp is suppressed completely (Held & Heup, 1996; Jiang & Struhl, 1996)), so Wg must start from zero there, causing it to ramp up so slowly perhaps that it gets swamped by the prevailing Dpp, hence forcing the outgrowth to wither soon after it begins.

Duplications do not pose the same converging versus diverging dilemma as the *triplications* because they typically grow to completion with hardly any tapering (Girton & Russell, 1980). Given that the complete (diverging) triplications come from the ventral (Wg) side of the disc, it should come as no surprise that virtually all of the duplications do also. Their etiology likely mimics that of diverging triplications (Figure 8e–g) with one exception. On the basis of the elements that vanish at the plane of symmetry, the area of apoptosis must occur *along the edge of the disc*, thus creating *only one Wg/Dpp interface*—and hence *only one extra leg*—instead of two (Figure 9).

Duplications and triplications that are remarkably similar to those evoked by *lethal(1)ts726* can be induced in wild-type leg discs by using a scalpel to partly bisect the disc on its ventral side, thus confirming that the injured area need not be entirely internal to trigger outgrowth (Bryant, 1971). Hence, the beetles and other hemimetabolous insects that were depicted in Bateson's book, which possess legs as juveniles, could have theoretically been goaded to triplicate by the kinds of external injuries (cuts or bites) that occur routinely in nature (cf. crustacean battle wounds (Shelton, Truby, &

FIGURE 10 Pacific chorus frogs (*Pseudacris regilla*) whose extra legs arose after the invasion of their hindlimb buds by parasitic flatworms during the tadpole stage. Above: Adult from Sheep Pond, Contra Costa County, California (Goodman & Johnson, 2011). Photo by Jeremy Monroe, Freshwaters Illustrated (Wikimedia Commons). Below: Adult from Aptos Pond, Monterrey County, California (Sessions & Ruth, 1990). Photo by Stan Sessions. On both its right and left sides this frog has one normal-looking hindlimb plus an extra pair of limbs located posteriorly. The extra limbs on the left side are completely separate distally but enclosed in a common sleeve of skin at the thigh area. The extra limbs on the right side are fused and enclosed in a common sleeve of skin throughout most of their length. The fused limbs on the right side are also distally hypomorphic (i.e., they lack a complete set of digits). Clearing and staining revealed that, in each case, the limb skeletons conform to Bateson's rule (L-R-L and R-L-R, respectively). On the frog's left side the digit formula (counting the normal-looking anterior-most limb) is 1–2-3–4-5/5-4-3-2-1/1–2-3–4-5, and on the right side it is 1–2-3–4-5/5-4-3/3–4-5

Shelton, 1981)) assuming, of course, that they utilize Dpp and Wg in roughly the same way as flies do. That assumption has been verified in crickets (Bando et al., 2018).

Further confirmatory evidence comes from cockroaches, where triplications can be elicited just by nicking the ventral (Wg) side of the leg (Bohn, 1965; Bryant et al., 1981; Shelton et al., 1981) or by transplanting right legs onto left stumps (or vice versa) (French et al., 1976) (Figure 2). Overall, therefore, the Dpp-Wg model that explains

Bateson's rule in flies appears to be generalizable to other insects (Angelini & Kaufman, 2005). Unfortunately, it does not apply to vertebrates, so we are forced back into the wilderness without a map, but as luck would have it, freaky frogs save the day.

7 | A PLAGUE OF EXTRA-LEGGED FROGS

In the 1990s, reports began to surface of frogs with extra legs in certain North American ponds (Blaustein & Johnson, 2003a; Souder, 2000)—up to a dozen legs per frog in some cases (Sessions & Ruth, 1990). Curiously, the multiple legs that sprouted from a common hip joint typically obeyed Bateson's rule—with left and right legs alternating successively in each such cluster (Figures 10 and 12). From a research standpoint, here was another golden opportunity (like the *lethal(1)ts726* mutant) to probe why extra legs are mirror-symmetric, but with the added bonus that the aberrant animals, in this case, were vertebrates.

Unlike insect legs, whose outgrowth relies on Dpp and Wg along the D-V axis, the arms and legs of vertebrates depend on different morphogens along the A-P axis. In salamanders, at least, the A and P morphogens are fibroblast growth factor-8 (Fgf8) and sonic hedge-hog (Shh). Recent experiments show that Fgf8 and Shh are both necessary and sufficient for salamander leg outgrowth (Figure 11).

Shh serves as the P morphogen for all vertebrate limbs, and the feedback loop between Shh and Fgf8 also appears to be universal (Zhu & Mackem, 2017). However, the exact role of Fgf8 varies among taxa: salamanders express it in their anterior mesenchyme (Tanaka, 2016), while birds and mammals express it in apical (distal) ectoderm within the apical ectodermal ridge (Vogt & Duboule, 1999).

Frogs appear to represent a compromise situation. In both *Xenopus laevis* (Yokoyama et al., 2000) and the direct-developing frog *Eleutherodactylus coqui* (Gross, Kerney, Hanken, & Tabin, 2011) Fgf8 is secreted apically (as in birds and mammals), but it is expressed more strongly on the A than the P side (as in salamanders), so it could indeed function as a polarizing agent along the A-P axis. Hence, the Fgf8-Shh model that has been proposed for salamander limb development (Figure 11) may also apply to frogs, allowing us to use this same circuitry in our analysis of extra-legged frogs.

The most likely explanation for the epidemics of deformed frogs seemed to be that affected ponds were polluted (Gilbert, 2001; Stocum, 2000), but researchers were gradually able to trace the extra-leg outbursts instead to population blooms of a tiny parasite that infests the limb buds of both tadpoles and salamanders (Sessions & Ruth, 1990). Whether these blooms are the result of ecological disturbances has yet to be determined (Johnson, Lunde, Zelmer, & Werner, 2003).

The parasite that is chiefly responsible for the regional epidemics is the flatworm *Ribeiroia ondatrae* (Johnson et al., 2006; Lunde & Johnson, 2012), first identified as the perpetrator in 1999 (Sessions, Franssen, & Horner, 1999). *R. ondatrae* has a life cycle with several intermediate hosts (Blaustein & Johnson, 2003b). It reproduces inside herons or egrets to produce eggs that enter ponds when the
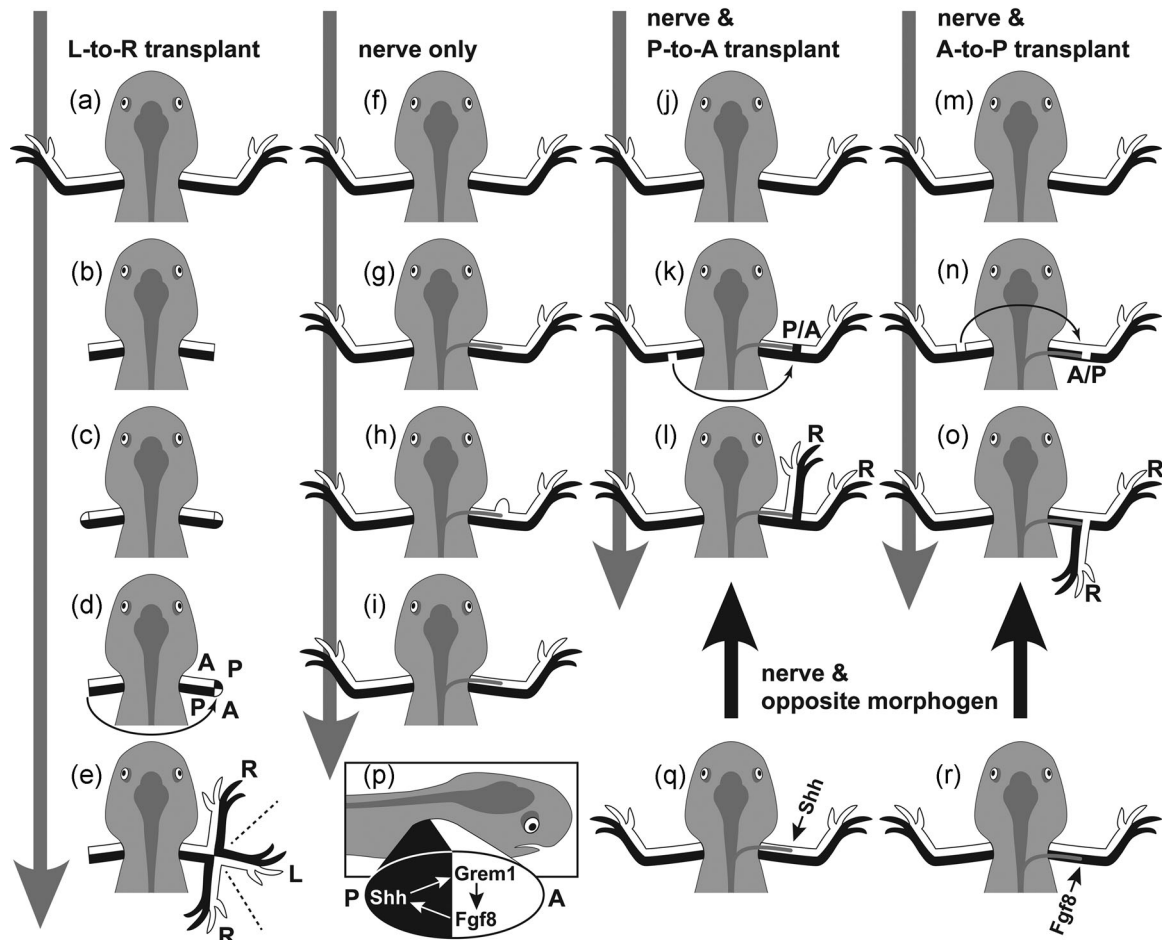
FIGURE 11 Unorthodox limb regeneration in adult salamanders. Each panel depicts the head and forelegs of a salamander. White and black mark anterior (A) and posterior (P) halves of the leg, with dark gray denoting brain and spinal cord. (a–e) Salamanders are exceptional among vertebrates for being able to fully regrow their appendages after reaching the adult stage (Tornini & Poss, 2014). The first step after amputation (b) is formation of a blastema of dedifferentiated, embryonic-like cells (c) (Alibardi, 2018; Haas & Whited, 2017). If the blastema of a left (L) foreleg is transplanted onto a right (R) stump (d), then a new foreleg grows out at each of the two A/P interfaces (e), due in theory to the same kinds of interactions that lead to triplications in salamander embryos (Figure 13j–l and p–r). (f–i) Rerouting a nerve into the A part of the forelimb (g) elicits a blastema (h) that regresses (i) (Mitogawa et al., 2018). (j–l) Doing the same plus inserting P tissue into the A region (k) causes an extra (R) limb to grow out (l). (m–o) The reciprocal operation—putting A tissue into nerve-supplied P region (n)—causes an extra (R) limb to grow out (o). (p) Blastema (embryo-like) limb field on a right stump (cf. (c)) in cross-section (oval) on a salamander viewed from the side, with black and white indicating P and A domains. Outgrowth is governed by a positive feedback loop between Shh and Fgf8 mediated by Grem1 (Tanaka, 2016; Zhu & Mackem, 2017). Grem1 belongs to a different (BMP) signaling pathway (Vogt & Duboule, 1999). (q–r) Proof that direct contact between A and P cells is not needed for limb outgrowth (Nacu, Gromberg, Oliveira, Drechsel, & Tanaka, 2016), as had been assumed in the polar coordinate model (French et al., 1976). The Shh protein can be used as a proxy for P cells (q), and the Fgf8 protein can be used as a proxy for A cells (r), leading in each case to limb outgrowth (l,o) (Nacu et al., 2016). Adapted from Nacu et al. (2016) and Tanaka (2016). BMP, bone morphogenetic protein; Fgf8, fibroblast growth factor-8; Grem1, gremlin-1; Shh, sonic hedgehog

birds defecate. The eggs hatch into first-stage larvae that infect snails and develop into second-stage larvae called "cercariae." The cercariae exit the snails and swim to tadpoles or salamanders, where they congregate in the pelvic folds (Stopper, Hecker, Franssen, & Sessions, 2002)—a posterior entry site that explains why hindlimbs are affected more than forelimbs (Johnson, Lunde, Ritchie, & Launer, 1999). Entry to forelimb primordia is prohibited by the covering of the gill pouch in frogs, but that is not the case for salamanders (Sessions & Ruth, 1990). After burrowing beneath the skin the larvae encase themselves in cysts and remain quiescent until the tadpoles metamorphose (Sessions & Ruth, 1990; Stopper et al., 2002). Any

resulting leg deformities will disable the frogs, making them easy prey for birds (Sessions & Ruth, 1990), thus repeating the cycle (Goodman & Johnson, 2011).

Conceivably, *R. ondatrae* might be inducing extra legs by secreting growth-stimulatory signals of some kind (Sessions & Ruth, 1990). However, their influence appears to be purely physical rather than chemical because uninfected tadpoles can be coaxed to sprout the same kinds of extra legs by just inserting inert beads into their limb buds (Sessions & Ruth, 1990). The resin beads that were used for this experiment were about the same size as the cysts themselves, with the diameter of each one being about
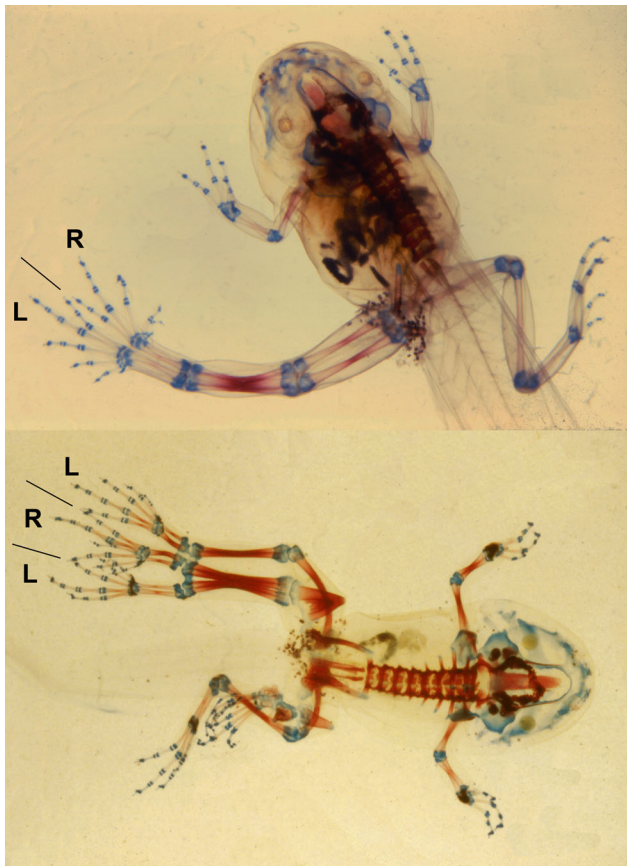
FIGURE 12 Frogs that exhibit planes of mirror symmetry (line segments) between their adjacent legs, with "L" and "R" denoting left- or right-handed legs (Sessions et al., 1999; Stopper et al., 2002). Both specimens are partly metamorphosed *Pseudacris regilla* frogs (note the tadpole tails), cleared and stained to reveal bones (red) and cartilage (blue). The dark dots peppering the pelvic area are cysts of the flatworm *Ribeiroia ondatrae*, each of which measures ~150 microns across (Sessions & Ruth, 1990). The cysts evidently caused the left leg bud of the upper individual to undergo an A-P duplication, yielding side-by-side legs (note the double femur). Both hindlimb buds of the lower frog sprouted extra legs. The left one made an A-P triplication with one whole leg plus a branched symmetric outgrowth. The mirror planes, which conform to Bateson's rule (see text), are clearly evident from the palindromic A-to-P digital sequences of the R/L legs above (5-4-3-2-1/1–2–3–4-5) and the L/R/L legs below (1–2–3–4-5/5-4-3-2-1/1–2–3–4-5), where numbers denote digits and slash marks are mirror planes. Most of the extra legs among infected frogs obey Bateson's rule, though this obedience is often not evident until the skin and muscle tissues are made transparent and the skeleton is stained with alizarin red (bone) and Alcian blue (cartilage), as shown here. Obedience to the rule along the D-V axis is nearly impossible to assess because the dorsal (upper) and ventral (lower) halves of the leg skeleton look virtually identical—for example, a left leg lying atop a right leg could not be distinguished from two apposed right legs. A-P, anterior-posterior; D-V, dorsal-ventral

a third the width of a hindlimb bud (Stopper et al., 2002). Dozens of parasites can wedge themselves into the vicinity of a single bud, and the resulting cysts remain behind in the hip region as the legs grow out, thus preserving unmistakable evidence of the original intrusion at the crime scene itself (Figure 12).

Heavy infestations can shred a bud into pieces like a shotgun blast, leaving cysts embedded throughout the area like shotgun pellets (Stopper et al., 2002). How this mangled mass of debris manages to recover enough to make anything recognizable is remarkable, let alone the fact that the legs that eventually sprout from the affected hip joints look relatively normal in both size and anatomy.

8 | OLD EXPERIMENTS SHED LIGHT ON NEW DATA

The capacity of the remaining cells to regroup into leg-forming islands amidst a sea of intruding cysts illustrates the versatility of vertebrate limb buds in general (De Robertis, Morita, & Cho, 1991). During their early stages they behave as self-organizing "embryonic fields" (Beloussov, Opitz, & Gilbert, 1997; De Robertis et al., 1991; Weiss, 1939), whose cells are not yet committed to form any specific part of the limb. The classic experiments that led to this conclusion were performed on salamander embryos by Ross Harrison (1870–1959) and published in 1918 (Harrison, 1918). For example, he found that each half of an incipient limb bud can make an entire leg in the absence of the other half (Figure 13a–f). He summarized the bud's properties as follows (boldface added):

> Self differentiating as the system is as a whole, the parts within the system do not constitute a developmental mosaic, with the exception of certain portions of the shoulder girdle. The system itself is equipotential, as shown by two tests to which it can be subjected; **a whole will develop out of a part**, and a single normal whole will develop out of two separate rudiments when fused together (Harrison, 1918).

Another experiment that Harrison conducted was to split a limb bud in half with a vertical incision. He expected each half to form a whole leg, just as they had done when their complementary demi-bud was excised, but a total of only one leg emerged from the bisected bud, apparently due to postoperative fusion of the halves to reconstitute the original field. Harrison's student F. H. Swett realized the problem and devised a way to prevent it. After cutting the bud in half, he pried open the wound and inserted a strip of flank skin that was incapable of participating in limb formation, thereby keeping the two halves a short distance apart.

Like Harrison, Swett expected each of his demi-buds to make a whole leg, and the front half-bud invariably did so, but the rear half-bud instead made *duplex* limbs that grew out as mirror-images of one another (Swett, 1926; Figure 13g–i). Overall, therefore, each of Swett's buds formed a total of three forelimbs in an R/L/R array, where the second slash mark represents not only a mirror plane but also the flank tissue wedged between the R anterior leg and the R/L duplicate behind it. This outcome mimics the L/R/L hindlegs of the lower frog in Figure 12, except that Swett studied *forelimb* buds on the *right* side of *salamanders*.
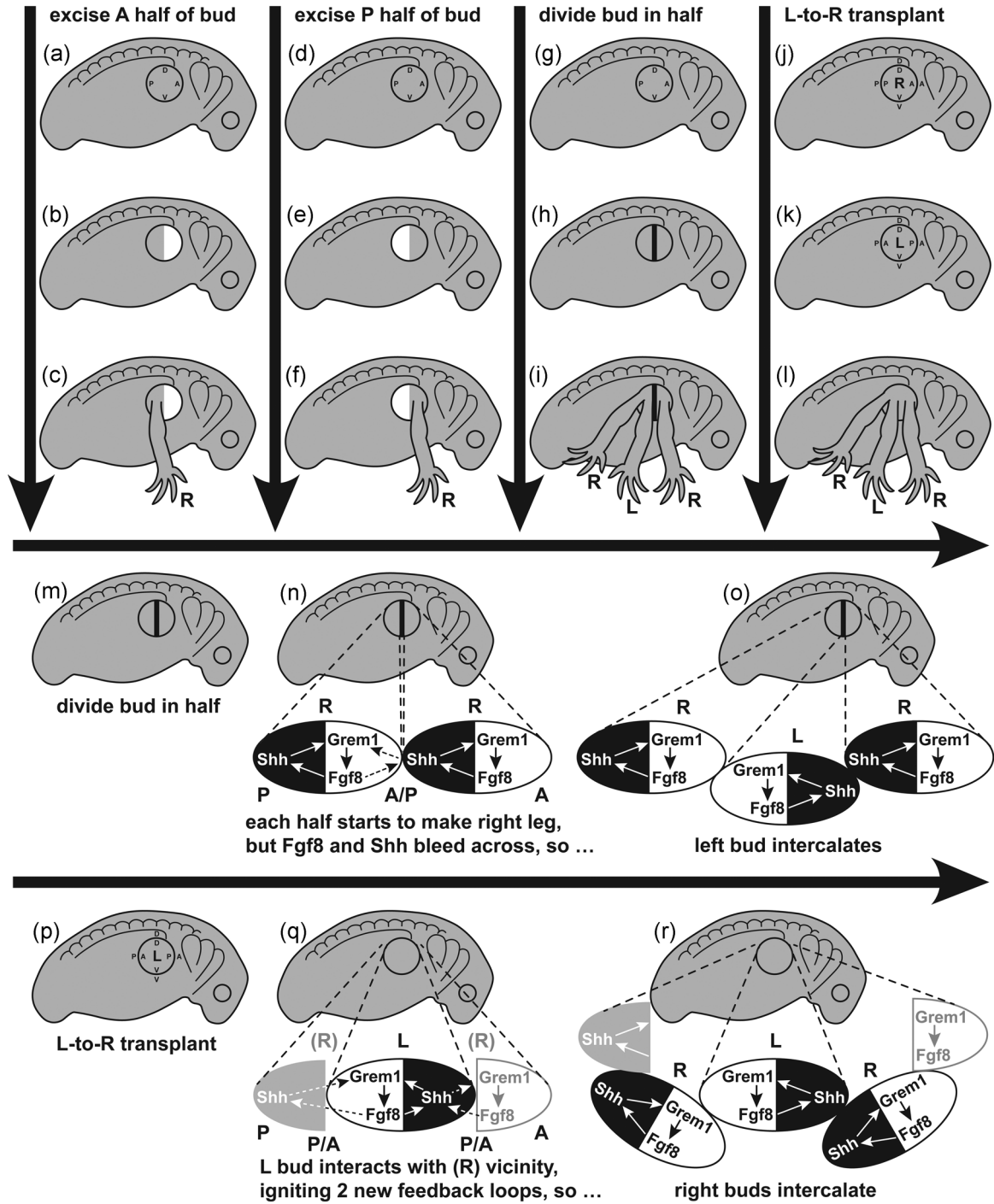
FIGURE 13 Continued.

Although the intervening strip was evidently wide enough to block the two halves from merging back together *physically*, it may have been too thin to stop the half-buds from influencing one another's responses *chemically* via molecular signals oozing across the isthmus (Figure 13m–o). Presumably, each half-bud started to make a right limb, but the nearness of the back part of the front limb to the front part of the rear limb could have elicited a left bud de novo between them (R/L/R).

This conjecture is not as farfetched as it may seem. Harrison conducted another series of experiments (published in 1921) whose results suggest this exact scenario. He excised most of the *right* forelimb bud of a salamander embryo and replaced it with a *left* forelimb bud. This surgery placed the left bud's back side at the front end of what had been the right bud and its front side at the rear end of that same area. The result of this confrontation was the outgrowth of triplicated R/L/R forelimbs (Figure 13j–l and p–r; Harrison, 1921), analogous to the symmetries seen after surgical experiments on adults that led to the polar coordinate model (Figure 2).

Given these themes from Harrison's and Swett's old experiments, it should come as no surprise that most of the legs growing from common hip joints in the parasite-infested frogs exhibited comparable planes of mirror symmetry. In those cases as well, neighboring buds may begin making legs of the same handedness, but ensuing interactions elicit intervening legs of opposite handedness (Figure 14). Not all of the legs growing out from the same hip joint attain the same length, possibly due to the chaotic nature of the parasitized bud. Large pockets of surviving cells will have a head start on small pockets, and neighboring clusters of opposite polarity may begin to fuse at different rates, depending in part on their relative sizes.

This patterning scenario is merely an extrapolation of Swett's experiment (Figure 13g–i), where each cyst would correspond to a strip of flank skin, though most buds would be diced repeatedly instead of being bisected singly. Frogs are as adept as salamanders at regenerating their limbs during the tadpole stage (Haas & Whited,

2017; Shimizu-Nishikawa, Takahashi, & Nishikawa, 2003), but they lose this ability as they mature (Mitogawa, Makanae, & Satoh, 2018).

9 | THE LOGIC OF MONSTERS

The most valiant attempt to decipher Bateson's rule ever published was made by his son Gregory (1904–1980) in 1971 (Bateson, 1971). The essay by the younger Bateson was published in the *Journal of Genetics*, which is fitting, of course, since Bateson Senior coined the term genetics in the first place. The core idea was that mirror planes between extra appendages are not merely analogous to the midline of a bilaterally symmetric animal but literally a default to that ground state due to their loss of some essential unit of information that would have allowed them to deviate from that state. This hypothesis is arguably more appropriate for another phenomenon his father is famous for naming (i.e., homeosis) since many such mutations are atavistic in causing a return to an evolutionary ground state (Lewis, 1994), but given what we now know about the extra-leg syndrome, it seems to miss the mark.

Despite their superficial differences, insects and vertebrates use many of the same genetic tools to build their legs. For instance, the hedgehog morphogen (known as Shh in vertebrates) establishes posterior identity in the appendages of both taxa. This genetic device has recently been shown to reign far beyond the arthropod and chordate realms: cephalopod mollusks also use hedgehog along the A-P axis of their appendages (tentacles)—but for *anterior* rather than posterior identity (Tarazona, Lopez, Slota, & Cohn, 2019). Such examples of "deep homology" (Held, 2017) suggest that insects and vertebrates might obey Bateson's rule because they use the same genetic circuitry to build their appendages. On the basis of the analyses presented here, however, we can see that this is not the case.

Rather, both taxa obey Bateson's rule for a more abstract reason. In each case, one of the Cartesian axes of their limbs uses a

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FIGURE 13 Pioneering experiments on salamander embryos. Panels depict the right side of a salamander embryo at the operative stage, with somites (arches), eye rudiment (tiny circle), and gills (petals) in the neck region. The forelimb bud (large circle) is inscribed with "compass" directions (D, dorsal; V, ventral; A, anterior; and P, posterior); the hindlimb bud is not yet visible. Adult forelegs (L and R are left and right) are drawn as if growing from the embryo even though they arise much later. Ovals (lower panels) denote embryonic limb fields, with black and white indicating P and A domains. (a–c) Removing the front half-bud does not result in half a leg. Instead, a whole leg emerges from the back half alone (Harrison, 1918). (d–f) Similar result when back half-bud is excised (Harrison, 1918). (g–i) Bisecting the bud and inserting a strip of (inert) flank skin (black rectangle) was expected to yield two legs, but it made three instead (Swett, 1926). Why does the middle (L) leg develop from the hind (vs. front) half? Probably because the rear half-bud has fewer limb-competent cells to start with than the front half-bud (Harrison, 1918), thus giving the latter a head start (Bateson, 1971) and allowing its diffusible signals to exert a greater influence across the gap. (j–l) Replacing the right forelimb bud with a left one reverses the A-P axis and yields three legs as in (i) (Harrison, 1921). (m–o) Hypothetical explanation for bud bisection results (g–i), based on the positive feedback loop (cf. Figure 11p) between Shh and Fgf8 that is mediated by Grem1 (Tanaka, 2016). Two extra (dashed) arrows have been added to the canonical circuit to indicate signals bleeding across the flank skin "barrier" at the A/P interface (n): Shh oozes posteriorly and Fgf8 oozes anteriorly. (p–r) Hypothetical explanation for L-R transplant results (j–l), based on new feedback loops that are sparked between adjacent limb fields at each P/A interface. The circuitry here only concerns the A-P axis, which was indelibly recorded by limb cells before the surgeries were performed, unlike the D-V axis, which was not specified or incorporated until later (Harrison, 1921). The latter axis relies on different signals from the A-P axis—namely, the Wnt pathway for the dorsal side and the BMP pathway for the ventral side (Delgado & Torres, 2017). The handedness or "chirality" of a limb (i.e., left vs. right) is defined by the relative orientations of these two axes, not by one axis alone. A-P, anterior-posterior; D-V, dorsal-ventral; Fgf8, fibroblast growth factor-8; Grem1, gremlin-1; Shh, sonic hedgehog
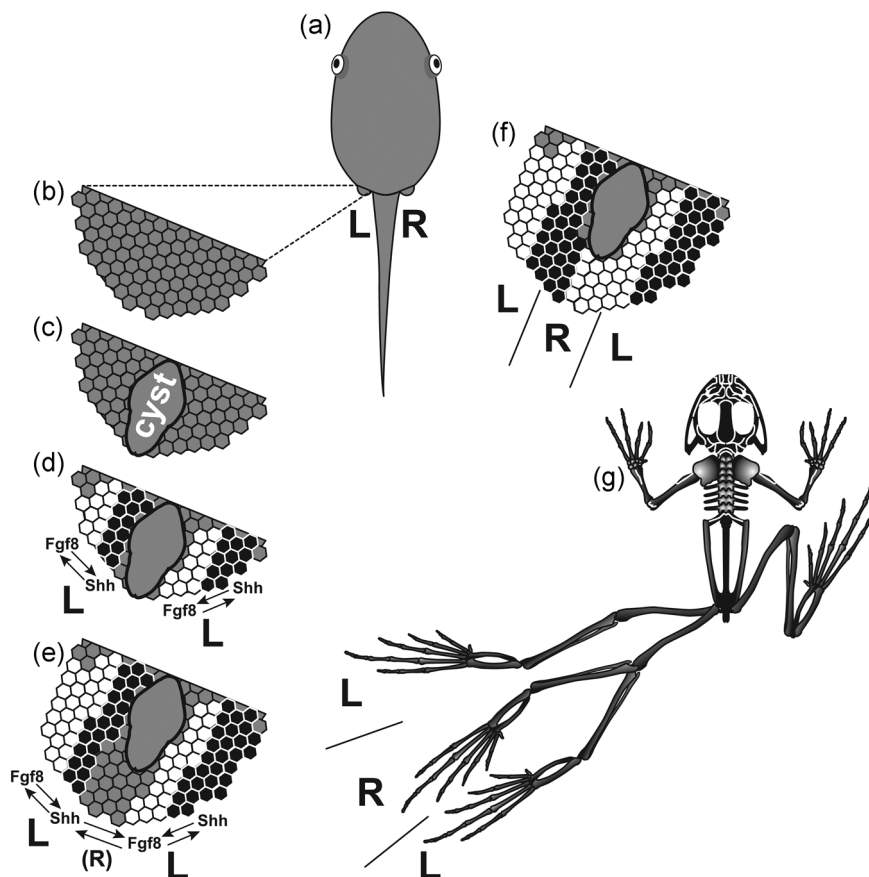
FIGURE 14 Hypothetical etiology of extra legs in parasite-infested frogs. (a) Tadpole, whose hindlimb buds are drawn as bumps that flank the tail (L, left; R, right). (b) Enlarged view of the L hindlimb bud, with cells shown as hexagons. Gray indicates uncommitted (pluripotent) embryonic cells. (c) A parasite has wedged itself into the center of the bud and formed an inert cyst. (d) Coalescence of cells into miniature limb fields on either side of the cyst. Black cells secrete Shh; white cells secrete Fgf8, and the two morphogens reinforce one another's expression by the positive feedback loop (arrows) that governs salamander limb development (Figure 11p; Grem1 has been omitted for clarity). (e) Outgrowth causes limb tissue to extend beyond the cyst, allowing morphogens from the two fields to bleed across the intervening space and ignite a Fgf8-Shh loop of opposite (R) polarity. (f) Continued outgrowth of these fields produces L/R/L legs that obey Bateson's rule. (g) Metamorphosed frog, with a bouquet of hindlegs on its left side. In this idealized rendition, the two rear members of the cluster are sketched as branching from a shared femur—the sort of phenotype often seen in parasitized frogs (Figure 12). The legs that end up fusing may depend upon the relative rates of mini-field outgrowth and the geometries of their contact angles when they grow beyond the cyst barriers. Whether such stochastic factors can explain the "nearer" versus "remoter" branches that Bateson witnessed in insects (Figure 1a) and cited in his rule remains to be seen. Readers who wish to tackle the latter problem should consult Jack Girton's exegesis of orthodromes, antidromes, and paradromes (Girton, 1981). Fgf8, fibroblast growth factor-8; Grem1, gremlin-1; Shh, sonic hedgehog

morphogen source at each end. Under normal circumstances, this bipolar device works well because each source is at the edge of the field and the morphogen has nowhere else to go but along the axis toward the other pole. The problem arises when more than one limb field tries to occupy the same space (due to parasites, injury, apoptosis, etc.). In that case, the diffusing morphogens can interact and create intervening dipoles of opposite polarity, leading to the L-R flip-flops of Bateson's rule. The mitotic spindle offers a good analogy because it has a similar geometry: each pole emits astral microtubules in all directions, but they are normally only needed in the direction of the other pole where chromosomes reside.

The actors obviously differ (Dpp-Wg in insects vs. Fgf8-Shh in vertebrates), as do the axes (D-V in insects vs. A-P in vertebrates), but the play remains the same. In both cases the feedback loops ensure that outgrowths are fueled by the ongoing morphogen interactions as self-organized, relatively independent modules. These loops therefore offer robustness in the face of injury-related deviations, but they harbor an Achilles heel. If an injury occurs in just the right place at just the right time, then the compensatory mechanisms that would normally correct the error (by wound healing) actually *overcorrect* and cause the system to career into a normally unoccupied valley of the epigenetic landscape (Saunders, 1990).

The resulting extra-leg deformities have no adaptive value, except perhaps for the gentleman Bateson mentioned whose double-hand helped him play piano. Hence, natural selection cannot be blamed: the reflection planes are an accidental side-effect of the system—that is, a "spandrel" (Gould & Lewontin, 1979). Pere Alberch

once wrote a lyrical tribute to such alternative anatomies, which look hideous to our eyes but seem perfectly normal to the participating cells because they are faithfully following the cues they encounter in their immediate neighborhood (Alberch, 1989). He aptly entitled his essay "The Logic of Monsters" (Diogo et al., 2017), and that same term certainly applies to the extra legs that obey Bateson's rule as well.

Positive feedback loops are easy to spark but hard to stop (cf. forest fires). Nevertheless, the extra legs of parasite-infested frogs typically reach the right size, as do the extra legs of insects (Heald, Hariharan, & Wake, 2015). How do limbs, or organs in general for that matter, manage to stop growing so precisely (Eder, Aegerter, & Basler, 2017; Hariharan, 2015; Vollmer, Casares, & Iber, 2017)? One idea for vertebrate limbs is that the poles of the feedback loop grow so far apart that they can no longer spur one another (Tanaka, 2016; Verheyden & Sun, 2008), but that trick cannot work for the Dpp-Wg loop due to their perpetual proximity. Alternatively, timers could be involved (Delgado & Torres, 2016), and evidence for their usage in vertebrate limbs has been adduced (Roselló-Díez, Arques, Delgado, Giovinazzo, & Torres, 2014; Saiz-Lopez et al., 2015). For cricket legs and maybe fly legs as well (Lawrence, Struhl, & Casal, 2008), the trigger for cessation appears to be the steepness of a proximal-distal gradient within each leg segment (Bando et al., 2009).

10 | EPILOGUE

The field of evolutionary developmental biology endeavors to decipher the rules that construct anatomy to understand how new species are sculpted from old ones (Hassan & Hiesinger, 2015). William Bateson had hoped to deduce the inner workings of developmental mechanisms by studying how they go awry, and his analyses of animal homeoses ultimately did prove successful in that regard (Lewis, 1994). Ironically, however, the very postulate that bears his name was far less fruitful. Bateson's rule has languished as a mere curiosity for more than a century, and now that the odd symmetries it codified have been somewhat demystified, their etiology appears to conform to everything we have learned from modern molecular biology.

Questions remain about the palindromic phenotypes. Chief among them is why mirror planes can subtend any angle between A-P and D-V, as illustrated by Bateson's rotating toy (Figure 1b). Do A-P and D-V axes leak signals from their poles to equal extents? What about the "nearer" versus "remoter" branches of a triplication described in the second axiom of Bateson's rule? What causes the uneven spacing? With regard to the branched-leg fly mutant, what is the nature of the mutation that links apoptosis to arc-shaped patches? How do duplicated hands arise in humans, and why is the mirror plane typically between the thumb and forefinger (Figure 1c)? If Swett's skin-strip experiments are repeated with a chemically impervious barrier, does only one leg grow out of each half bud, and does the chirality always match? If two right limb buds are

transplanted side-by-side at a remote (flank) site in a salamander embryo, does an extra left leg always grow out between them?

Arguably, Bateson's rule is telling us something important about the resilience of pattern-forming mechanisms and the plasticity of limb development. And it offers at least an intimation regarding possible constraints on limb evolution.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study—regarding the frog specimens depicted in Figures 10 and 12 in particular—are available from Stanley K. Sessions upon request.

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