of flexion. In anterior view, the envelope had greatest height sagittally, and greatest width in pure lateral flexion (compare Tables 1 and 2). Laterally, *Apatosaurus* could curl its neck to form a tight U-curve, with its head extended laterally over 4 m, owing to the large, flat zygapophyses of the proximal cervicals. The zygapophyseal shape in *Diplodocus* permitted less angular deflection laterally, but the longer neck resulted in similar total lateral deflection of about 4 m.

The primary difference between these two genera was in dorsiflexion. The maximum feeding height was about 6 m for *Apatosaurus* compared to 4 m for *Diplodocus*. The shorter yet more flexible neck of *Apatosaurus* could have achieved a pose close to the high S-curve in which it is frequently depicted, whereas *Diplodocus* was barely able to elevate its head above the height of its back. The zygapophyseal design of *Apatosaurus* permitted greater decoupling between flexion in the horizontal plane and in the vertical plane. At the extremes of lateral flexion, *Apatosaurus* still had sufficient flexibility to feed from ground level up to more than 3 m, essentially squaring off the upper and lower corners of the feeding envelope. In contrast, lateral flexion of *Diplodocus* resulted in cumulative dorsiflexion distally, raising the head about 2 m above ground level in maximum lateral flexion, whereas the head of *Apatosaurus* could be at or near ground level with the same lateral flexion. Thus, the feeding envelope of *Apatosaurus*, in anterior view, was subrectangular (both high and low feeding at the lateral extremes), whereas that of *Diplodocus* was more diamond-shaped, with less vertical range permitted at the lateral extremes and less overall elevation. Surprisingly, the necks of both genera were capable of more ventriflexion than dorsiflexion, reaching at least 1.5 m below ground level.

The low browsing envelopes of *Diplodocus* and *Apatosaurus*, coupled with their similarities in skull shape and dentition (*27*), suggest that these two sympatric giants may have been feeding on the same kinds of vegetation. The abundance of both genera in Morrison deposits suggests either that both were generalized feeders or that the plants on which they fed were so abundant that competition between the two genera was not a factor. Despite the similarities in feeding envelopes and cranial morphology of the two diplodocids, *Diplodocus* had a longer neck (6.2 m versus 5.3 m) and was more gracile in body shape and vertebral design than *Apatosaurus*, which had roughly three times the mass.

The extreme ventral flexibility in diplodocids is beyond the mobility required for quadrupedal feeding. Their ability to ventriflex sharply would have allowed a diplodocid on the margin of a lake or river to extend its head outward and downward to graze on plants on or under the water. Alternatively, some workers (*7–9*) have advocated tripodality in diplodocids, and a tripodal *Diplodocus* could have fed from 6.3 to 11.6 m off the ground. However, the upper-canopy arborescent plants available for a high-feeding sauropod in the Late Jurassic consisted primarily of conifers and ginkgoes (*28–31*), neither of which would provide particularly nutritious forage for large herbivores like diplodocids (*32*). On the other hand, an abundance of soft, high-nutrition vegetation—in the form of ferns, cycadeoids, seed ferns, horsetails, and algae—was available to terrestrial sauropods feeding along the shores of perennial lakes and rivers (*33*), a scenario for which the great neck length and the ability to flex ventrally and traverse a broad lateral arc would have been advantageous. Moreover, the presence of a near horizontal neck in diplodocids renders moot the problem of supplying blood to an elevated sauropod brain. Rather than flexing their necks like dinosaurian counterparts of giraffes or swans, they appear to have fed more like giant, long-necked bovids.

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Morphological Clues from Multilegged Frogs: Are Retinoids to Blame?

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Morphological analysis was performed on multilegged deformed frogs representing five species from 12 different localities in California, Oregon, Arizona, and New York. The pattern of duplicated limbs was consistent with mechanical perturbation by trematode infestation but not with the effects of retinoids.

Reports of amphibians with supernumerary limbs from natural populations have been in the scientific literature for centuries, but the causes remain unclear (*1–3*). Recent reports of deformed frogs from Minnesota (*4*) and elsewhere have generated a resurgence of interest. Suggested causes include environmental pollution, ultraviolet irradiation (*3, 5*), genetic mutation, microbes, parasites, and some other disease (*2, 6*). Most reported malformations involve missing or extra hind limbs (*2–4*). Missing limbs are difficult to inter-

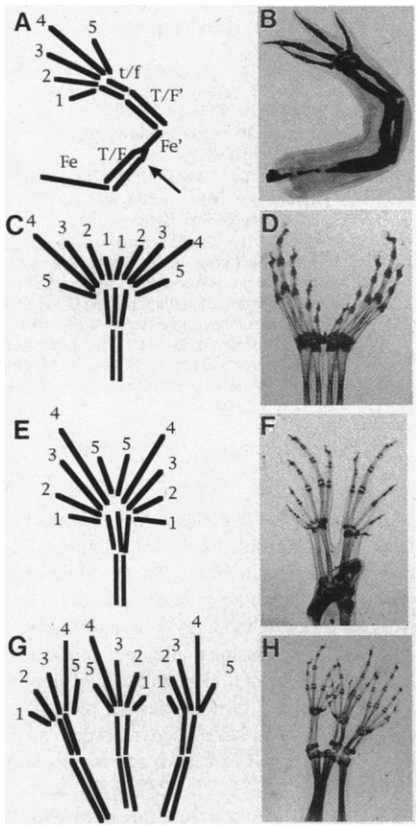

Fig. 1. Mirror-image limb duplications (**A, C, E, G**) (digits are numbered from anterior to posterior) and actual mirror-image duplications seen in an RA-treated forelimb of *A. mexicanum* (**B**) and field-caught multilegged *H. regilla* treefrogs (**D, F, H**). (A and B) PD duplication (Fe = femur, T/F = tibia/fibula, Fe' = serially duplicated femur, T/F' = serially duplicated tibia/fibula, t/f = tibulare/fibulare; arrow indicates amputation site through the tibia/fibula); (C and D) PMID; (E and F) AMID; (G and H) MIT.

pret, but supernumerary limbs often contain important morphological clues to the underlying mechanism (*2, 7*).

Supernumerary limbs can be experimentally induced in tadpoles by treatment with retinoids such as retinoic acid (RA) or by mechanical perturbation of developing or regenerating limbs (*8–12*). The possible involvement of retinoids in recent reports of deformed amphibians is of interest because retinoids interact with steroid hormone-like receptors and include some of the most powerful known human teratogens (*9*). One common pesticide is a suspected retinoid mimic (*13*). The effects of retinoids and mechanical perturbation on amphibian limbs allow predictions of specific kinds of morphological abnormalities (*2, 7, 8–12*). By comparing the morphological patterns found in multilegged amphibians from natural populations with these predicted patterns, it should be possible to identify the most parsimonious explanation for this kind of deformity.

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Table 1. Limb duplications observed in multilegged Pacific treefrogs (*H. regilla*) examined in this study. PDD = proximal-distal duplication. Each deformity is expressed as percent of total number of mirror-image duplications observed. *n* = number of specimens examined. Last row is a summary of limb duplications produced by RA treatment of three species of amphibians (*10, 11*).

Locality	Date	*n*	AMID	Deformity PMID	MIT	PDD
Aloha, OR	1997–1998	2	0	2	0	0
Aptos, CA	1987/98	314	41	59	24	0
Corvallis, OR	1998	12	4	5	1	0
Grants Pass, OR	1997	2	0	2	0	0
Jackson County, OR	1997–1998	7	1	1	0	0
La Pine, OR	1997	52	8	11	7	0
Roseburg, OR	1997	1	1	0	0	0
Umatilla, OR	1997	1	0	1	0	0
Total		391	55 (32.2%)	81 (47.4%)	35 (20.5%)	0 (0%)
RA		128	0 (0%)	10 (7.8%)	1 (0.78%)	117 (91.4%)

RA inhibits intact developing limb buds and causes two kinds of duplications in regenerating limbs: serial duplications along the proximal-distal axis (PD duplications) (Fig. 1, A and B) and, less commonly, mirror-image duplications along the anterior-posterior axis (*14*). RA biochemically changes the positional values of cells by inducing ectopic gene expression in all three primary axes of the limb bud: anterior-to-posterior, dorsal-to-ventral, and distal-to-proximal (*9, 15–17*). RA-induced mirror image duplications in the anterior-posterior axis are always double posterior mirror-image duplications (PMIDs) (Fig. 1C); this is true even in RA-induced ectopic limbs from amputated tails of frog tadpoles (*18*). Likewise, RA proximalizes distal cells (*17*), which explains the predominance of PD limb duplications in experimentally treated amphibians (*19, 20*).

Limb duplications can also be produced by any mechanical perturbation that causes physical rearrangement of cells in the developing or regenerating limb (*2, 7, 12, 21*). Surgical rotation of limb buds in amphibians, for example, results in the outgrowth of several limbs from one original limb bud (*7, 12*). A less dramatic result can be obtained by moving small groups of cells between different regions within the limb bud (*22*). The effects of such mechanical perturbation include double anterior mirror-image duplications (AMIDs) (Fig. 1E), as well as PMIDs, mirror-image triplications (MITs) (Fig. 1G), and "bony triangles" (*12*). Notably absent from the range of effects of mechanical perturbations are PD duplications.

The results of a morphological analysis of 391 specimens of cleared and stained multilegged Pacific treefrogs (*Hyla regilla*) from eight different localities are summarized in Table 1. We have also examined small samples of multilegged specimens of four other anuran species: cascades frog (*Rana cascadae*) from Oregon, wood frog (*Rana sylvatica*) from New York, green frog (*Rana clamitans*) from New York, and leopard frog (*Rana pipiens*) from Arizona. All these specimens are infested with trematode cysts located in and around the deformities (*23*).

The supernumerary limbs and related deformities in these samples of frogs are confined to the hind limbs in all but five of the specimens. The total number of hind limbs ranges from 1 to 12. The observed morphological patterns (Table 1) include a wide range of deformities—bony triangles (Fig. 1F) and both kinds of anterior-posterior mirror-image duplications, PMIDs (Fig. 1D) and AMIDs (Fig. 1F), as well as MITs (Fig. 1H). In addition, about 1686 specimens of long-toed salamanders (*Ambystoma macrodactylum*) of various ages were found with similar limb deformities at the same site that yielded large numbers of multilegged *H. regilla* (*2*). Not a single example of a PD duplication was found in any of these specimens. Similar results have recently been obtained from a sample of more than 300 multilegged *H. regilla* (*24*). These observed morphologies are not consistent with the effects of retinoids on amphibian limbs, but they are within the range of limb abnormalities expected from mechanical perturbation (Table 1). Recent studies have shown that trematode cyst infestation in the laboratory can induce the outgrowth of supernumerary limbs in amphibians (*25*). These studies, combined with the evidence presented here, argue against retinoids as an explanation for the apparent increase in reports of this kind of deformity in natural populations. Instead, evidence points to the need for additional research on environmental factors that affect trematode infestation rates.

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The Effect of Trematode Infection on Amphibian Limb Development and Survivorship

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The causes of amphibian deformities and their role in widespread amphibian declines remain conjectural. Severe limb abnormalities were induced at high frequencies in Pacific treefrogs (*Hyla regilla*) exposed to cercariae of a trematode parasite (*Ribeiroia* sp.). The abnormalities closely matched those observed at field sites, and an increase in parasite density caused an increase in abnormality frequency and a decline in tadpole survivorship. These findings call for further investigation of parasite infection as a cause of amphibian deformities in other sites and species.

Alarm over increasing reports of deformed amphibians has intensified since the early 1990s (*1, 2*). Over the last decade, abnormalities have been reported in 36 species of amphibians from 42 U.S. states (*3*). Whether abnormalities are contributing to global trends in amphibian population decline or are indicative of environmental threats to human health is still uncertain (*1, 4, 5*). Suggested causes of abnormal amphibians include ultraviolet-B radiation, biocide contamination, retinoids, and parasite infection (*1, 6–9*). However, none of these have been decisively linked to the types of abnormalities most frequently reported in the field: missing, malformed, and extra limbs (*3, 7, 10*).

Between 1996 and 1998, we surveyed 35 ponds in Santa Clara County, California, to determine the prevalence of abnormal amphibians. At 4 of the 13 ponds supporting Pacific treefrogs, severely abnormal frogs were observed. Intensive monitoring programs established at two of these ponds consistently recorded high frequencies (15 to 45%) of metamorphic frogs with polymely (extra limbs) and other hindlimb deformities (n = 8818; Table 1). Water tests failed to detect any pesticides, polychlorinated biphenyls (PCBs), or heavy metals, and 200 *H. regilla* eggs collected from the ponds hatched and developed normally in the laboratory (*11*). Community analysis of the 35 ponds revealed that the four ponds with abnormal treefrogs were the only ponds to support both *Hyla regilla* and an aquatic snail, *Planorbella tenuis*, which is a first host of the trematode parasite *Ribeiroia* sp. Upon dissection, we found *Ribeiroia* metacercariae in treefrogs from each of the four ponds. Whereas three other trematode species were also observed in some of the dissections, *Ribeiroia* exhibited a unique distribution within infected frogs: the metacercariae were highly localized in the tissue around the pelvic girdle and hindlimbs, often in close association with abnormal or extra limbs.

We tested the hypothesis that *Ribeiroia* infection is responsible for the limb abnormalities that we observed in *H. regilla*. The experiment operated within an ecologically relevant framework by exposing tadpoles to living parasites at observed field densities, allowing cercariae to freely select a point of penetration, and employing an amphibian host species for which high abnormality rates have been recorded in the field. We collected *H. regilla* egg masses from the Eel River (39°44′N, 123°39′W), an area 300 km north of our monitoring sites with no known records of abnormal frogs (*12*). After hatching, tadpoles were kept individually in 1-liter containers of commercial spring water and randomly assigned to one of six treatments. Those in the experimental treatments were exposed to either 0 (control), 16 (light), 32 (intermediate), or 48 (heavy) *Ribeiroia* cercariae. A fifth group was exposed to 80 cercariae of a second species of trematode (*Alaria mustelae*) also found in frogs from Santa Clara County field sites. The sixth group was exposed to both species: 80 *Alaria* cercariae and 32 *Ribeiroia* cercariae (*13*). Infection levels were selected to encompass the range of parasite densities found in naturally infected abnormal frogs collected from our field sites. Tadpoles were exposed to parasites in four equal doses over a 10-day period, with each dose equal to one-fourth of the total parasite load (*14*).

Exposure of Pacific treefrog tadpoles to *Ribeiroia* cercariae induced severely abnormal limb development in 85% of the frogs surviving to metamorphosis (n = 71). The frequency of abnormalities was high in all *Ribeiroia* treatments and showed a positive relationship to parasite density (logistic regression $\chi^2 = 88.16$, df = 3, $P < 0.001$; Fig. 1A). Tadpole survivorship declined with increasing parasite load and fell below 50% in the intermediate and heavy treatments (logistic regression $\chi^2 = 29.86$, df = 3, $P < 0.001$; Fig. 1A). In the control group, 88% of the tadpoles survived, and all survivors were normal. Only 40% of the tadpoles in heavy treatment survived to metamorphosis and

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