

Morphological and Physiological Correlates of Hybrid Parthenogenesis

Michael Kearney* and Richard Shine†

School of Biological Sciences A08, University of Sydney, New South Wales 2006, Australia

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ABSTRACT: Asexuality frequently evolves in association with hybridity and polyploidy. The phenotypic consequences of this association must be understood before we can fully appreciate the reason for the short-term success, and the long-term failure, of asexual genetic systems. Parthenogenetic forms within the Australian gecko complex *Heteronotia binoei* are triploid hybrids and overlap geographically with both of their parental taxa as well as another sexual lineage. We compared nine morphological and nine physiological traits in wild-caught adult sexual and asexual individuals as well as their captive-bred progeny across a 1,200-km latitudinal gradient. Genome dosage effects were apparent in that the parthenogens were most phenotypically similar to the parental form for which they had a double dosage. Physiological differences between parthenogens and sexuals were often in the opposite direction of that expected under heterosis. Sympatric populations of sexual and parthenogenetic *H. binoei* differ in ecologically significant phenotypic traits, possibly reducing niche overlap. In keeping with the Red Queen hypothesis, parthenogenetic *H. binoei* had more ectoparasitic mites than did sexuals in some regions. Lizards with high mite loads also had high rates of evaporative water loss, suggesting a direct link between phenotypic traits and vulnerability to parasites.

Keywords: parthenogenesis, hybridity, polyploidy, morphology, physiology, ectoparasites.

Asexual animals frequently arise through hybridization. This appears to have been the case for all parthenogenetic

vertebrates that have been studied in detail (Vrijenhoek et al. 1989; Avise et al. 1992; Adams et al. 2003) and many parthenogenetic invertebrates (White et al. 1977; Montovani and Scali 1992; Johnson and Bragg 1999; Delmotte et al. 2003). Changes in ploidy also are common correlates of the evolutionary transition to asexual reproduction and are often the result of further hybridization events with sexual relatives (e.g., Parker and Selander 1976; Johnson et al. 1999). A close association between asexuality, hybridity and polyploidy is also well known in plants, the so-called agamic complexes (Stebbins 1950, 1971). Agamic complexes of animals and plants show significant ecological biases in their distributions, occurring most frequently at high latitudes and altitudes, in xeric environments, on islands or island-like environments, and in “disturbed,” “marginal,” or “ecotonal” environments (Stebbins 1950, 1971; Wright and Lowe 1968; Glesener and Tilman 1978; Bell 1982; Kearney 2003).

Why is there such a strong link among the genetic phenomena of asexuality, hybridity, and polyploidy, and what causes their peculiar ecological associations? Many studies of agamic complexes have emphasized the significance of the transition from sexual to asexual genetic systems. This transition leads to all-female populations that exhibit clonal reproduction, a potentially twofold increase in population growth rate, and an enhanced colonizing ability (Wright and Lowe 1968; Maslin 1971; Williams 1975; Cuelar 1977; Glesener and Tilman 1978; Maynard Smith 1978; Bell 1982). Such profound changes will clearly have important ecological implications. But what is the significance of hybridity and polyploidy? Hybridity may provide the genetic disturbance necessary to allow the origin of asexuality (Wetherington et al. 1987; Moritz et al. 1989a), and polyploidy may provide an important means to stabilize newly arisen asexual lineages (Bell 1982). If these are the only roles of hybridity and polyploidy, then they could be regarded as epiphenomena to asexuality.

However, there are many reasons to expect that asexual organisms evolving in association with hybridization and polyploidy will differ phenotypically from their parental forms (Lynch 1984). Additive interactions among the

* Corresponding author. Present address: Centre for Environmental Stress and Adaptation Research, La Trobe University, Bundoora, Victoria 3086, Australia; e-mail: m.kearney@latrobe.edu.au.

† E-mail: rics@bio.usyd.edu.au

newly combined genomes may produce intermediate phenotypes in the asexual hybrids relative to their sexual progenitors (the intermediate niche hypothesis; Moore 1977; Bulger and Schultz 1982; Schlosser et al. 1998). There also may be nonadditive interactions among the combined genomes (Otto 2003). For example, hybrid asexuals have very high, fixed heterozygosity, which may lead to more vigorous and broadly tolerant phenotypes (the spontaneous heterosis hypothesis; Schultz 1971; Bulger and Schultz 1979, 1982; Moore 1984; Wetherington et al. 1987; Schlosser et al. 1998). Alternatively, negative interactions among the newly combined genomes may produce intrinsically poorer phenotypes relative to those of the progenitors (the outbreeding depression hypothesis; Cullum 1997). Polyploidy also may have phenotypic effects stemming from an increase in cell size and nuclear content (Stebbins 1950, 1971; Szarski 1970, 1983; Zhang and King 1993; Soltis and Soltis 2000). Finally, multiple hybrid origins can provide a source of clonal diversity, and selection among clonal lineages thus may cause divergence between the phenotypes of asexual and sexual forms, potentially favoring "general purpose genotypes" (Parker et al. 1977; Lynch 1984) or specialized genotypes (Vrijenhoek 1979, 1984).

Such phenotypic changes may directly influence the distribution patterns of asexual organisms; they may even be of primary importance (Suomalainen 1962; White 1970; Schultz 1971; Cole 1975; Mitton and Grant 1984; Beaton and Hebert 1988). Moreover, if asexual lineages are phenotypically divergent from their progenitors, the subsequent reduction in niche overlap may permit varying degrees of coexistence between them. Thus, if we are to understand the reasons for the success of asexual organisms in certain environments and their degree of coexistence with sexual forms, we must also understand the phenotypic consequences of their hybrid origins and of polyploidy.

This challenge requires accurate identification of the sexual progenitors of asexual lineages (Darevsky et al. 1985). In this study, we compare a suite of phenotypic traits among parthenogenetic and sexual forms of the Australian gekkonid lizard *Heteronotia binoei*, a case of hybrid, polyploid parthenogenesis. The origin of parthenogenesis in this complex has been thoroughly studied throughout its distribution using chromosomes, allozymes, nuclear ribosomal RNA genes, and mitochondrial DNA (mtDNA; summarized in Moritz 1993). Briefly, the parthenogenetic races originated through hybridization events between two chromosomally distinct sexual races, CA6 and SM6 (Moritz 1983, 1984; Moritz et al. 1989b). The initial hybridization events are proposed to have created diploid parthenogenetic hybrids, CA6/SM6, with subsequent backcrossing events leading to the formation of triploid

races CA6/SM6/CA6 and CA6/SM6/SM6 (forms A and B/C, respectively, in the original terminology [Moritz 1983]). In gross morphology, these two triploid forms resemble the sexual parental form for which they have a double genetic dosage. That is, CA6/SM6/CA6 has a banded back pattern like the CA6 sexual race, and CA6/SM6/SM6 has a speckled back pattern like the SM6 sexual race. The two parthenogenetic forms inhabit some of the driest regions of the Australian arid zone, where they are broadly sympatric with three of the sexual forms, including the two progenitor sexual races as well as the EA6 sexual race (fig. 1).

Here we compare nine morphological and nine physiological traits between the two triploid races *H. binoei* (CA6/SM6/CA6 and CA6/SM6/SM6) and the CA6, SM6, and EA6 sexual races. We use wild-caught adults and their captive-bred progeny in these comparisons. First, we consider phenotypic differences between parthenogenetic races of *H. binoei* and their sexual progenitors. This comparison addresses the evolutionary issue of how the phenotypes of parthenogenetic forms have changed relative to their sexual ancestors. Second, we compare the phenotypes of parthenogenetic *H. binoei* with sympatric sexual populations. This comparison addresses the ecological issue of the likely degree of niche overlap among sympatric parthenogenetic and sexual populations of *H. binoei*. Third, we address the potential phenotypic effects of parasite load. Parthenogenetic organisms often have higher parasite loads than sympatric sexual relatives, a pattern consistent with the Red Queen hypothesis for the advantage of sex (e.g., Lively et al. 1990; Moritz et al. 1991). Parthenogenetic *H. binoei* have more ectoparasitic mites (genus *Geckobia*) than do sympatric sexual forms (Moritz et al. 1991), and this may have phenotypic consequences, particularly for physiological traits. Thus, we have tested for associations between physiological traits and mite burdens in sexual and parthenogenetic lineages.

Methods

Collection and Maintenance of Animals

We sampled geckos from 22 sites along a 1,200-km latitudinal gradient through central Australia between August 22 and September 4, 2000, spanning a region where the 3N1 maternal lineage (Moritz 1991) of parthenogenetic *Heteronotia* is broadly sympatric with the EA6, CA6, and SM6 sexual races (fig. 1; sites are presented in table A1 in the online edition of the *American Naturalist*). We obtained sexual individuals from 14 of these sites and parthenogenetic individuals from 17, with actual syntopy of sexual and parthenogenetic races at nine sites (fig. 1). Identity of the lizards was confirmed (after data collection was

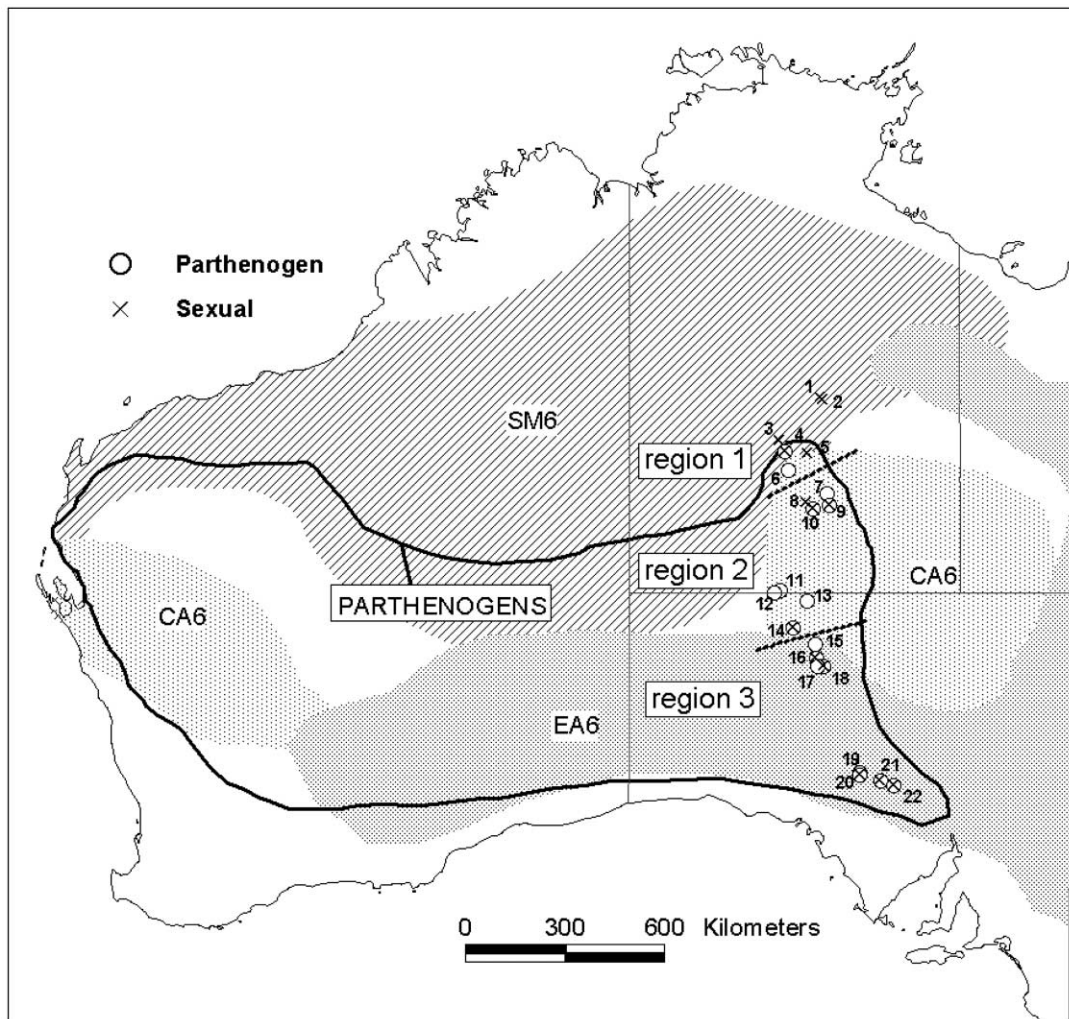


Figure 1: Distribution of parthenogenetic *Heteronotia binoei* (heavy outline) in relation to the three sexual forms with which they coexist (shaded), as well as collecting sites of parthenogenetic and sexual individuals used in this study. Numbers refer to localities in table A1 in the online edition of the *American Naturalist*. The three regions used in the “ecological” comparisons are also indicated and are separated by the dotted lines.

complete) by genetic typing using a combination of mtDNA sequencing and microsatellite markers (J. L. Strasburg, in preparation).

We housed specimens in male/female pairs (sexuals) or female/female pairs (parthenogens) in a controlled environment room at the University of Sydney. We incubated eggs produced by the captive animals in the laboratory and maintained hatchlings under identical conditions to the wild-caught lizards (for more details on animal housing and egg incubation, see Kearney and Shine 2004). An additional 13 female CA6 hatchlings, derived from females captured from Bond Springs and Undoolya in March 1998, were also included in the analyses. These individuals were incubated and measured at the same time as hatchlings

obtained from individuals collected in 2000, and there were no differences between CA6 hatchlings from these two sources for any of the morphological or physiological traits considered (all $P > .25$).

Measurement of Morphological and Physiological Traits

We took the following morphological measurements for each lizard immediately upon collection from the wild: mass, snout-vent length (SVL), axilla-groin length (AGL), tail length, head length (HL), head width (HW), the number of supra- and infralabial scales, and fluctuating asymmetry of the labial scales. We measured head length from the anterior edge of the tympanum to the tip of the snout,

and we measured head width at the widest part of the head. Labial scales were counted for both sides of the face and then averaged to obtain a value for each individual. Fluctuating asymmetry was calculated as the absolute value of the difference in the total number of labial scales between the right-hand and left-hand sides of the face (Van Valen 1962). We also counted the number of small red mites (*Geckobia* sp.) visible on each lizard upon collection from the wild (September 2000) and at two subsequent times (March 2001 and November 2001). All morphological traits (except AGL and HL) were measured for captive-bred lizards immediately upon hatching (Kearney and Shine 2004).

We measured the following physiological traits for each adult lizard: critical thermal minimum (CT_{min}), critical thermal maximum (CT_{max}), voluntary thermal minimum (VT_{min}), voluntary thermal maximum (VT_{max}), selected body temperature (T_{sel}), variability of body temperature (T_{var}), evaporative water loss rate (EWL), standard metabolic rate (SMR), and burst running speed. The CT_{min} and CT_{max} are the lower and upper temperatures, respectively, at which the righting response is lost. We measured the CT_{min} in this way, but to ensure the safety of the animals, we recorded the CT_{max} as the panting threshold. The VT_{min} , VT_{max} , and T_{sel} are the minimum, maximum, and median temperatures selected in a laboratory thermal gradient over a 24-h period, while T_{var} was the standard deviation in body temperature over this time. As an indication of thermal niche breadth, we calculated the difference between the CT_{max} and CT_{min} (critical thermal niche breadth, CT_b) and between the VT_{max} and VT_{min} (voluntary thermal niche breadth, VT_b). We also made measurements of the CT_{min} , CT_{max} , CT_b , EWL, and SMR for captive-bred juvenile lizards. Burst running speeds of hatchlings have already been reported in Kearney and Shine (2004). All measurements of wild-caught animals were made after at least 50 days in captivity. All measurements of captive-bred lizards were made within 300 days of hatching. A detailed description of the methods used to measure the physiological traits is provided in appendix B in the online edition of the *American Naturalist*.

Data Analysis

We compared mean trait values among taxa using ANOVAs and ANCOVAs. For the “evolutionary” comparisons of the two progenitor sexual races, CA6 and SM6, with the two chromosome races of parthenogen, CA6/SM6/CA6 and CA6/SM6/SM6, only female individuals of these races were used, and the EA6 sexual race was excluded. A two-way ANOVA was then performed with reproductive mode and genome dosage as factors. For this

analysis, each trait was tested with and without body mass as a covariate, except for SVL and scalation traits. We compared wild-caught adult lizards as well as captive-bred hatchling lizards to examine the likelihood that the differences observed had a genetic basis. For the “ecological” comparison between parthenogens and the sexuals with which they co-occur, we assigned the 22 populations to three broad regions according to the distributional ranges of the three sexual races (fig. 1). A two-way ANOVA was then performed with reproductive mode and region as factors. The factor reproductive mode included parthenogenetic females, sexual females, and sexual males as levels. For these analyses, we used body- and head-size traits as well as physiological traits but excluded the scalation traits, since they are less likely to be of ecological relevance. Finally, we used repeated measures ANOVA to test for differences in mite load between parthenogenetic and sexual female lizards across the three geographic regions, and we then correlated the physiological traits with mite load and with each other. Mite loads for this comparison were assessed at three different times during the study (September 2000, March 2001, and November 2001). Some traits were log transformed to improve equality of variances. All post hoc comparisons are Tukey adjusted to control for multiple comparisons. Because data on scalation as well as fluctuating asymmetry of scalation violated the assumptions of parametric ANOVA, we used the equivalent nonparametric Kruskal-Wallis test incorporating the extension developed by Scheirer et al. (1976; as described in Sokal and Rohlf 1995, p. 445).

Results

For brevity, we summarize here only those results that were statistically significant. Trait summary statistics and sample sizes can be found in tables C1 and C2 in the online edition of the *American Naturalist*, while summaries of statistical analyses can be found in tables C3–C10. Selected comparisons also are shown graphically in figures D1–D5 in the online edition of the *American Naturalist*.

“Evolutionary” Comparisons

Two-way ANOVA and ANCOVA for morphological traits indicated significant genome dosage effects for snout-vent length ($F = 8.54$, $df = 1, 138$, $P = .004$), mass-adjusted head width ($F = 38.60$, $df = 1, 137$, $P < .0001$), mass-adjusted head length ($F = 28.41$, $df = 1, 137$, $P < .0001$), and the number of supralabial scales ($H = 10.38$, $df = 1, 138$, $P = .001$) and infralabial scales ($H = 16.22$, $df = 1, 138$, $P < .001$), with lizards biased to the CA6 genotype (CA6 sexuals and CA6/SM6/CA6 parthenogens) being smaller with narrower heads and having a greater

number of labial scales than those biased to the SM6 genotype (SM6 sexuals and CA6/SM6/SM6 parthenogens; fig. D1a–D1c). There were also significant reproductive mode effects: parthenogenetic lizards had larger mass-adjusted heads than sexual forms (head width: $F = 5.56$, $df = 1, 137$, $P = .020$; fig. D1b; head length: $F = 7.72$, $df = 1, 137$, $P = .006$) and had more infralabial scales ($H = 5.73$, $df = 1, 137$, $P = .017$). For the mass-adjusted analysis of AGL, there was a significant interaction whereby parthenogens had smaller mass-adjusted AGLs than sexuals, but this effect was strongest for CA6-biased lizards (interaction term: $F = 5.74$, $df = 1, 137$, $P = .018$).

Two-way ANOVA and ANCOVA for physiological comparisons of wild-caught adults indicate genome dosage effects for critical thermal minima (CT_{min} ; $F = 28.94$, $df = 1, 130$, $P < .0001$), critical thermal maxima (CT_{max} ; $F = 4.05$, $df = 1, 130$, $P = .046$), mass-adjusted evaporative water loss rates (EWL; $F = 7.32$, $df = 1, 120$, $P = .008$), and mass-adjusted standard metabolic rates (SMR; $F = 9.81$, $df = 1, 125$, $P = .002$); CA6-biased lizards had lower values for CT_{min} , EWL, and SMR than did SM6-biased lizards and higher values for CT_{max} (figs. D1, D3a). Lizards biased to the CA6 genotype also had a greater critical thermal niche breadth (CT_b ; $F = 23.60$, $df = 1, 127$, $P < .0001$; fig. D3a). Reproductive mode contrasts indicated wild-caught parthenogens had lower CT_{min} ($F = 6.21$, $df = 1, 130$, $P = .014$), CT_{max} ($F = 4.32$, $df = 1, 130$, $P = .040$), and selected body temperatures (T_{sel} ; $F = 5.18$, $df = 1, 127$, $P = .025$) than their sexual progenitors (fig. D3a). Parthenogens also had higher mass-adjusted evaporative water loss (EWL) than sexuals ($F = 4.25$, $df = 1, 120$, $P = .042$; fig. D1d). There was an interaction between reproductive mode and genome dosage for the voluntary thermal minimum (VT_{min} ; $F = 14.07$, $df = 1, 128$, $P < .001$) and mass-adjusted voluntary thermal niche breadth (VT_b ; $F = 9.97$, $df = 1, 127$, $P = .002$) whereby the CA6 females had significantly lower VT_{min} and a broader VT_b than SM6 females and the two parthenogenetic forms (fig. D3a). An interaction was also apparent for burst running speed ($F = 11.37$, $df = 1, 120$, $P = .001$), with SM6 females running faster than CA6 females and the two parthenogenetic forms (fig. D1f).

Physiological comparisons of captive-bred juveniles indicated genome dosage effects for CT_{min} ($F = 7.09$, $df = 1, 95$, $P = .009$) and mass-adjusted CT_b ($F = 7.17$, $df = 1, 90$, $P = .009$), with CA6-biased lizards having lower CT_{min} and broader CT_b than SM6-biased lizards (fig. D3a). Parthenogenetic hatchlings had lower mass-adjusted CT_{max} than did sexual hatchlings ($F = 7.15$, $df = 1, 121$, $P = .009$; fig. D3a) and higher EWL ($F = 4.21$, $df = 1, 81$, $P = .043$; fig. D1d). A significant interaction between reproductive mode and genome dosage was appar-

ent for SMR ($F = 5.61$, $df = 1, 101$, $P = .020$); hatchling parthenogens tended to have higher metabolic rates than hatchling sexuals; this effect was weaker for SM6-biased lizards (fig. D1e).

“Ecological” Comparisons

The “ecological” comparisons of morphology between sympatric parthenogenetic and sexual *Heteronotia binoei* showed significant effects of reproductive mode for log body mass ($F = 14.08$, $df = 2, 196$, $P < .0001$), log head width (HW; $F = 11.70$, $df = 2, 196$, $P < .0001$), and log axilla-groin length (AGL; $F = 6.25$, $df = 2, 196$, $P = .002$). Post hoc Tukey’s tests reveal that, across all three geographic regions, parthenogens had a greater body mass and head width than did male or female sexuals (and sexual females had a greater body mass than males; fig. D2a, D2b). Males also had significantly smaller AGL than did parthenogens and sexual females. Interactions were present between reproductive mode and geographic region for log SVL ($F = 2.62$, $df = 4, 196$, $P = .036$) and log HL ($F = 5.03$, $df = 4, 195$, $P < .001$); parthenogenetic females had greater SVL and HL than sexual males and females in region 3 (and sexual females had greater SVL than males), parthenogens had greater SVL than sexual males and greater HL than sexual males and females in region 2, while in region 1 there were no differences in SVL and males had longer heads than parthenogens. When the comparisons were made with body mass as a covariate, sexual females were found to have relatively smaller heads than both males and parthenogens. In addition, there was an interaction between geographic region and reproductive mode for head length, with parthenogens having relatively longer heads than males and females in region 3, parthenogens and males having relatively longer heads than sexual females in region 2, and males having relatively larger heads than parthenogens in region 1.

For physiological traits, the “ecological” comparisons show reproductive mode effects for CT_{min} ($F = 8.61$, $df = 2, 184$, $P < .001$) and CT_{max} ($F = 7.06$, $df = 2, 182$, $P = .001$). Post hoc Tukey’s test indicated that parthenogenetic and sexual females had higher CT_{min} than males and that parthenogenetic females had lower CT_{max} than sexual males and females across all geographic regions (fig. D3b). A reproductive mode effect was also evident for CT_b ($F = 9.90$, $df = 2, 178$, $P < .0001$), with parthenogens significantly narrower than sexual males and females (fig. D3b). Interactions between reproductive mode and geographic region complicated the patterns for VT_{min} ($F = 2.90$, $df = 4, 180$, $P = .023$), VT_b ($F = 2.57$, $df = 4, 180$, $P = .040$), EWL ($F = 3.65$, $df = 4, 169$, $P = .007$), SMR ($F = 3.78$, $df = 4, 179$, $P = .006$), and burst running speed ($F = 3.07$, $df = 4, 169$, $P = .018$). Parthe-

nogenetic females and sexual males had higher VT_{\min} and narrower VT_b than sexual females in region 2 only (fig. D3b). Parthenogenetic females had greater water loss rates than sexual females in region 3 and males in region 2, but there were no differences in region 1 (fig. D2c). The metabolic rates of parthenogens were lower than those of sexual males and females only in region 1 (fig. D2d). Finally, the burst running speed of parthenogenetic females was slower than that of sexual females in regions 1 and 3 (fig. D2e). Although mass was a significant covariate for some traits (CT_{\max} , VT_{\min} , VT_{\max} , and EWL), the only conclusion that changed by adjusting for body size was that parthenogenetic females had greater EWL than sexual females and males in region 3.

Mite Load and Physiological Correlations

Repeated measures ANOVA of mite load, with reproductive mode and geographic region as factors and time of mite count as the repeated measure, indicated a significant interaction between reproductive mode and geographic region ($F = 5.28$, $df = 2, 106$, $P = .007$). Separate comparisons of parthenogens to sexuals for each region indicated that parthenogens had higher mite counts than sexuals only in region 3 (region 3, $P < .001$; region 2, $P = .273$; region 1, $P = .186$; fig. D4). Mite loads of lizards also increased over the three time periods (within-subjects time effect; $F = 12.45$, $df = 2, 106$, $P < .0001$; fig. D4).

There were few significant correlations among the physiological traits we measured, and they were all among the traits measured in the thermal gradient (VT_{\min} , VT_{\max} , T_{sel} , and T_{var}). Evaporative water loss was the only physiological trait correlated with mite load (fig. D4), and this occurred for all three mite count dates, although EWL measurements were made closest to the March 2001 count (September 2000 mite count, $R = 0.44$, $P < .001$; March 2001 count, $R = 0.44$, $P < .001$; November 2001 mite count, $R = 0.36$, $P = .002$).

Discussion

Genome Dosage Effects and Geographic Patterns

The simplest phenotypic outcome of a hybrid origin of parthenogenesis is for the newly combined genomes to act additively and produce intermediate phenotypes in the parthenogenetic forms relative to the sexual progenitors. In cases of polyploidy, we would expect phenotypic similarity to reflect the dosage summations of the parental genomes, as appears to be true for morphology and behavior in a number of unisexual vertebrates (Schultz 1969; Menzel and Darnell 1973; Thibault 1974; Moore 1984).

In the case of *Heteronotia binoei*, we would expect CA6/SM6/CA6 parthenogens to most closely resemble the CA6 parental form and CA6/SM6/SM6 parthenogens to most closely resemble the SM6 parental form. In addition to the gross back pattern effects already stated, our study found such genome dosage effects for snout-vent length (SVL), head width (HW), head length (HL), supra- and infralabial scales, critical thermal minimum (CT_{\min}), critical thermal maximum (CT_{\max}), evaporative water loss rates (EWL), and standard metabolic rate (SMR) for wild-caught adult lizards (figs. D1, D3a). A genetic basis to these patterns is supported by the persistence of some of these differences in captive-bred juvenile lizards in the laboratory: SVL and infralabial scales (Kearney and Shine 2004), CT_{\min} and critical thermal niche breadth (CT_b ; fig. D3a), as well as two additional traits: body mass and labial scale fluctuating asymmetry (Kearney and Shine 2004; although we cannot completely exclude the possibility of maternal effects or ontogenetic changes for hatchling data).

Genome dosage effects also occur in *H. binoei* with respect to the climatic environments occupied by CA6/SM6/CA6 and CA6/SM6/SM6 parthenogens from both the 3N1 maternal lineage (considered in this study) and the 3N2 maternal lineage (Kearney et al. 2003). These maternal lineages represent independent origins of parthenogenesis in *H. binoei*. Analysis of mtDNA sequences has localized the origins of parthenogenetic *H. binoei* to geographically restricted areas in western Australia, with subsequent eastward and southward expansion (Moritz 1991; Moritz and Heideman 1993). If polyploidy evolved close to the point of origin (as suggested by the high frequency of western-type allozymes in the parthenogenetic lineages: Moritz et al. 1989b), the two triploid forms (CA6/SM6/CA6 and CA6/SM6/SM6) must have invaded (or persisted in) different climatic conditions during this range expansion. Genome dosage effects with respect to morphology and physiology including those demonstrated in this study therefore may explain these distributional patterns and thus may have contributed to the ecological breadth of parthenogenetic forms. For instance, CA6 sexual lizards and CA6-biased parthenogenetic lizards (CA6/SM6/CA6) occur in cooler environments (Kearney et al. 2003) and also have lower critical thermal minima.

Nonadditive Effects: Evidence for Heterosis?

In addition to genome dosage effects, we also observed overall differences between the phenotypes of parthenogens and sexuals (i.e., reproductive mode effects in the ANOVAs). Traits affected in this manner for wild-caught adult lizards were HW, HL, infralabial scale counts, CT_{\min} , CT_{\max} , T_{sel} , and EWL (figs. D1, D3a). These effects persisted into the second generation for CT_{\max} and EWL (figs. D1,

D3a), suggesting a genetic basis, and additional effects of reproductive mode on body mass and SVL in hatchlings already have been shown in a previous study (Kearney and Shine 2004). We also observed more complex cases where the effects of reproductive mode interacted with those of genome dosage. For example, both adult (this study) and juvenile (Kearney and Shine 2004) parthenogenetic *H. binoei* of the CA6/SM6/CA6 and CA6/SM6/SM6 lineages had low burst running speeds similar to those of the CA6 sexual race but were slower than the SM6 sexual race (fig. D1f).

These effects of reproductive mode and the interactions between reproductive mode and genome dosage presumably reflect the outcome of polyploidy and/or nonadditive interactions among the CA6 and SM6 genomes within the parthenogens, including dominance and/or epistatic effects. We also note, however, that some degree of genome dosage by reproductive mode interaction may actually be expected under a simple additive model because, unless there is full dominance of one genome over the other, the difference between CA6/SM6/CA6 and CA6/SM6/SM6 polyploids should be less than that between CA6 and SM6 sexuals. Nonadditive phenotypic consequences of a hybrid origin also have been observed in morphological comparisons of diploid and triploid parthenogenetic *Aspidoscelis* (formerly *Cnemidophorus*) *tesselatus* and their sexual parental forms (Parker 1979). In the latter study, non-additive effects were seen in the diploid and triploid forms, which indicates that they were not solely due to the effects of polyploidy per se. Since no diploid parthenogenetic *H. binoei* are known, however, we cannot assess the relative influences of hybridity and polyploidy on the patterns we observed.

Do our data support the hypothesis that parthenogenetic hybrids exhibit heterosis or “hybrid vigor” (Bulger and Schultz 1979), or do they instead suggest outbreeding depression (Cullum 1997)? Heterosis is certainly a possibility in *H. binoei* since parthenogenetic forms have a much higher mean heterozygosity ($H = 0.32$) than their sexual progenitors (CA6, $H = 0.06$; SM6, $H = 0.09$; Moritz et al. 1989b). Indeed, parthenogenetic *H. binoei* have among the highest levels of heterozygosity of any vertebrate (Dessauer and Cole 1989). If heterosis was present, we might expect to see evidence for a larger size and a more robust and broadly tolerant physiology in the parthenogens. In agreement with this prediction, adult parthenogens had relatively larger heads than sexuals (this study) and juvenile parthenogens hatched at larger body sizes (Kearney and Shine 2004). We note, however, that these effects may also reflect the effects of polyploidy on cell size (Stebbins 1971; Szarski 1983). With respect to the whole-organism physiological traits we measured, we might expect to see faster running speeds, broader thermal tolerances (Bulger and Schultz 1979; Bulger and Schultz

1982), lower standard metabolic rates (Mitton 1993), and lower water loss rates in parthenogens relative to sexuals (Mitton and Grant 1984). In addition, lower metabolic rates are also predicted in polyploids (Szarski 1983), and greater cold and heat tolerance has been observed in polyploid *Artemia* (Zhang and Lefcort 1991). None of these predictions were met. Instead, parthenogens were slower (although no slower than the CA6 sexual race) and had narrower thermal niches (although were generally more cold tolerant), higher water loss rates, and, at least in juveniles, higher metabolic rates. Similar patterns were reported in a broad study of physiological traits among five independent origins of parthenogenesis in the teiid lizard genus *Aspidoscelis*, where parthenogens had slightly increased EWL and SMR, slightly decreased burst running speed, and significantly reduced endurance (Cullum 1997). Another physiological comparison of sexual and parthenogenetic *Aspidoscelis* found that they had similar thermal preferences but that the sexual race thermoregulated with greater precision (Sievert and Paulissen 1996).

It is difficult to assess whether the physiological differences between parthenogenetic and sexual *H. binoei* represent outbreeding depression resulting from interactions among the newly combined genomes, the accumulation of mutations as predicted by Muller’s ratchet (Muller 1964), or interclonal selection among the original suite of clones produced by hybridization (Vrijenhoek 1979; Lynch 1984; Parker and Niklasson 2000). If the latter case was true, we could not regard parthenogenetic *H. binoei* as physiologically inferior to the sexual forms but simply different as a result of different selection histories. High water loss rates and metabolic rates, for instance, are not necessarily deleterious but may instead reflect an alternative ecological strategy. Parthenogenetic *H. binoei* are biased toward cooler climates (Kearney et al. 2003), and this study as well as a previous one (Kearney and Shine 2004) suggest parthenogenetic *H. binoei* are better adapted to cold. Considerable insight could be gained into the underlying causes of the patterns we observed if it proves possible to artificially produce parthenogenetic *H. binoei* by hybridization experiments in the laboratory (e.g., White et al. 1977; Wetherington et al. 1987; Hotz et al. 1999).

Ecological Implications of Phenotypic Differences

The distribution of parthenogenetic *H. binoei* overlaps with three different sexual forms: the two sexual progenitors CA6 and SM6 as well as the EA6 sexual race (fig. 1). Irrespective of the ultimate reasons for the phenotypic differences we observed between parthenogenetic *H. binoei* and their sexual progenitors, such differences may have significant implications for ecological interactions among sympatric taxa. The regional “ecological” comparisons re-

vealed significant differences between each sexual race of *H. binoei* and the parthenogenetic forms occurring sympatrically with them. For example, parthenogenetic *H. binoei* consistently were larger than sympatric sexual forms in body and head size and had lower critical thermal maxima (figs. D2, D3b). The voluntary thermal minimum, variability of body temperature, evaporative water loss rate, standard metabolic rate, and burst running speed also differed between parthenogens and sexuals in one or more regions (figs. D2, D3b).

Differences in body and head size have strong implications for dietary niche partitioning (e.g., Roughgarden 1972; Schoener 1974), and the physiological differences may reflect differences in the fundamental niches of these lizards with respect to the times and places they can forage in as well as the kinds of retreat sites they can choose (Kearney and Porter, in press). These phenotypic differences may therefore reduce niche overlap between parthenogenetic and sexual *H. binoei* and thus increase their potential for coexistence. Reduction in niche overlap may also be mediated by the extensive clonal diversity of *H. binoei* (Moritz et al. 1989b). The frozen niche variation hypothesis proposes that ecological differences among clones lead to differences in resource use that allow multiple clones, as well as sexual forms, to stably coexist (Vrijenhoek 1978, 1979, 1984). We found significant differences in morphology and physiology between CA6/SM6/CA6 and CA6/SM6/SM6 clones of *H. binoei*, possibly contributing to their ability to coexist at many sites, despite an absence of distributional overlap among the sexual EA6, CA6, and SM6 races (fig. 1). Moreover, preliminary analyses of microsatellite-based clones within each triploid form of *H. binoei* indicate significant morphological and physiological differences among clones (M. Kearney, J. L. Strasburg, and R. Shine, unpublished data).

Interplay between Ectoparasite Load and Physiology

Moritz et al. (1991) found that wild-caught parthenogenetic *H. binoei* had a significantly greater burden of ectoparasitic mites (genus *Geckobia*) than did sympatric sexual populations. These mites are visible to the naked eye and can form dense colonies on the geckos, particularly around the perimeter of the eye and in the groin area. Our data on mite load show the same pattern, especially in region 3 (fig. D4). Our data also demonstrate that the higher burden of parasites in the parthenogens persists after many months in captivity.

Moritz et al. (1991) pointed out that this pattern is consistent with the Red Queen hypothesis for the advantage of sex, whereby an absence of genetic recombination in the parthenogenetic forms renders them more susceptible to parasitic attack. However, they did not know

whether or how parasitic mites affect the fitness of the geckos. We previously found no relationship between mite load and the reproductive output of parthenogenetic or sexual *H. binoei* (M. Kearney and R. Shine, unpublished manuscript). However, the current study revealed a strong positive relationship between evaporative water loss rate and mite load (fig. D5). This leads to two intriguing and potentially nonexclusive possibilities. First, the mites may increase water loss rates through tissue damage or through consumption of bodily fluids; this would represent a clear detrimental effect of mites on gecko fitness and further support the Red Queen hypothesis. Second, geckos with high water loss rates may somehow constitute a better "habitat" for the mites, allowing them to reach higher densities. In the latter case, parthenogenetic geckos may have higher water loss rates as a result of outbreeding depression, for instance, and as a secondary consequence also have a high parasite burden. This presents a clear alternative to the Red Queen explanation and is worthy of further experimental attention, especially in view of the fact that the captive-bred (and therefore mite-free) parthenogenetic geckos had higher water loss rates than their sexual progenitors (fig. D1d).

In conclusion, our results indicate that significant phenotypic change has accompanied the transition from sex to parthenogenesis via hybridization within the *H. binoei* complex, with potentially important consequences for the geographic distributions of the parthenogenetic lineages and their ecological interactions with sexual forms. It is crucial to determine the relative role of such phenotypic changes and that of parthenogenesis per se in the short-term success of hybrid parthenogenetic organisms before we can fully interpret their relevance to the problem of sex.

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