

Review

Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination¹

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Abstract

The mechanisms that control growth and reproduction have received considerable attention by molecular and cellular endocrinologists, yet there has been relatively little effort to link these two aspects of physiology. On the other hand, evolutionary biologists have long commented on the relationship between growth and reproduction in many species, yet have generally neglected the mechanisms underlying such complex traits. An approach that integrates the multiple proximate levels promises to provide significant insight into the evolution of neuroendocrine control mechanisms. In this chapter, we take this approach in reviewing environmental influences on growth and reproduction in the leopard gecko, *Eublepharis macularius*. In this species, incubation temperature during embryonic development not only determines gonadal sex, but also underlies within-sex differences in growth, adult morphology, aggressiveness, reproductive physiology and behaviour, and brain organization. Thus, the leopard gecko is an excellent model to elucidate the developmental interactions among the environment and the endocrine and nervous systems that control growth and reproduction. © 1998 Elsevier Science Inc. All rights reserved.

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1. Introduction

Biologists who study different levels of organization tend to ask different questions. For example, molecular and cellular biologists and physiologists ask how environmental effects on growth and reproduction are mediated by endogenous mechanisms within the organism (i.e. proximate causation) whereas evolutionary biologists ask why these effects occur (i.e. ultimate causation) [2]. Can these questions be combined in a

manner that may elucidate the evolutionary context of the problem at hand? For example, what forces of selection act to shape the mechanisms that mediate environmental effects on growth and reproduction? An integrative and comparative approach must be used to fully answer this type of question.

Such an approach has become important as we recognize that development is a process marked by the emergence of new structural and functional properties arising through reciprocal interactions among already existing constituents at all levels of analysis (molecular, organismic and environmental) [33]. This complexity has increased with the discovery that organizing features can be transmitted across generations epigenetically. For example, transfer of maternal steroids and other hormones to the developing embryo have a pro-

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found effect on the phenotype of the offspring [1,3,7,69]. In canaries and zebra finches, eggs laid on later days have higher testosterone (T) levels than eggs laid earlier which, in turn, correlates positively with the subsequent growth and social rank of the individual; i.e. males hatched from eggs laid later tend to grow faster and achieve higher social status [70,71]. As would be expected, circulating levels of T, which increase during yolking, vary among females and under different environmental regimens [72]. The spotted hyena displays similar, but more pronounced, maternal effects on offspring phenotype. In this species, the female's clitoris is hypertrophied, resembling the male's penis. This unique pattern of female urogenital development results from maternal ovarian androstenedione production that is transformed to testosterone in the placenta and subsequently transported to the fetus. Other aspects of the female's phenotype, such as body size and aggressive behavior, are also masculinized such that females dominate males in the social group [32].

Such modifying factors need not be derived solely from the mother, but can arise from the architecture of pregnancy. In mammals, including humans, the position of the fetus relative to its siblings can mold its morphology, reproductive physiology and sociosexual behaviour in adulthood [14,15]. For example, it is now well-known that in gerbils, as in mice and rats, female fetuses located between two males (2M females) are exposed to higher levels of androgens produced by the neighboring males compared to female fetuses located between two females (2F females) [80]. As adults, 2M females have lower oestradiol (E2) and higher T levels in the circulation, have a masculinized phenotype and are less attractive to males and more aggressive to females. 2M females also produce litters with more male-biased sex ratios relative to 2F females [13]. Additionally, 2M females have later onsets of oestrous cycles, longer oestrous cycles, shorter reproductive lives and fewer litters [13]. Differences in phenotype due to intrauterine position are also evident in males. Males positioned next to two males (2M males) are more aggressive and less sexually active than males positioned next to two females (2F males). It should be noted, however, that the pattern of intrauterine effects are not necessarily consistent across species. For example, in gerbils 2M males outcompete their 2F counterparts in reproductive success, whereas in mice 2F males are more sexually active than their 2M brothers [13].

The metabolic activity of brain nuclei, as measured using cytochrome oxidase (COX) histochemistry, also vary according to uterine position (see below for more detailed discussion on COX). The metabolic capacity in the sexually dimorphic area of the preoptic area (SDA-POA) and the posterior portion of the anterior hypothalamus is greater in 2M than 2F females [42]. In gerbils, the SDA-POA is responsible for copulatory

behaviour in males [83], and the posterior portion of the anterior hypothalamus is an area replete with neurons containing gonadotropin releasing hormone [76]. These differences in metabolic capacity may explain, respectively, the behavioural and hormonal masculinization of 2M females relative to 2F females.

One of the more interesting aspects of intrauterine position effects is the transmission of phenotypes. As stated above, 2M females tend to have litters with more male-biased sex ratios. This means that the female offspring of such females are more likely to be 2M females than are the female offspring of 2F females. The same is true for male offspring (i.e. males are more likely to have male fetuses as neighbors). Therefore, through hormonal effects in utero, the life histories of females may be transmitted nongenomically.

In humans, the cochlea produces spontaneous otoacoustic emissions, and the frequency of such emissions can be influenced by the prenatal environment. In general, females produce more emissions than males. This sexual dimorphism, however, is not evident in the female of opposite-sex fraternal twins, suggesting that the prenatal environment created by the male fetus decreases these emissions in such females [51,52].

Finally, the experiences of the female while pregnant or even the behaviour of the mother towards the neonate can influence how the individual grows and behaves. Indeed, more than 30 years ago Christian and LeMunyan [12] demonstrated that the adverse effects of crowding mice during pregnancy can continue to be detected in the physiology and behaviour of the following two generations of progeny. Similar stress effects can be induced by handling pregnant females or housing them in socially unstable conditions [37,64,81]. Lastly, Moore et al. [53,54] discovered that mother rats behave differently toward male and female pups and, further, these differences reinforce and accentuate subsequent sex differences when the pup reaches adulthood.

Thus, the transmission of traits across generations can occur both by the inheritance of genes from parent to offspring as well as by non-genomic means. These studies highlight the need to address issues as the environment before birth and the social interactions between the mother and immature offspring in the study of individual differences. It is noteworthy that in all of these examples the genetic sex and gonadal sex of the individual are linked, making it difficult to distinguish environmental from genetic contributions. For example, to what extent are the differences observed between adult males and females due to their differences in sex chromosomes, differences in non-genomic yet heritable traits, the nature and pattern of hormone secretion, or even sex-typical experiences? To tease apart these elements, we have been studying an animal model system that allows us to dissociate the effects of

gonadal sex (i.e. sex steroid hormones) from that of environmental variables. Here we review our research on the leopard gecko, *Eublepharis macularius*, to illustrate how the environment interacts with the developing endocrine and nervous systems to cause variation in growth and reproduction. We emphasize not only the proximate mechanisms underlying phenotypic differences, but also the selection pressures that may have influenced the evolution of such mechanisms.

2. The leopard gecko: a non-mammalian model for environmental effects on growth, reproduction and neuroendocrinology

The laboratory studies the long-term effects of embryonic incubation temperature in the leopard gecko, a species in which temperature determines gonadal sex during the middle of embryonic development [8,9]. This mode of sex determination is widespread in reptiles: all crocodylians, most turtles and many lizards display temperature-dependent sex determination or TSD [24,41,45,47]. In this species, incubation of eggs at 26°C produces only female hatchlings (low-temperature), at 30°C produces a female-biased sex ratio (approximately 25% male) (female-biased incubation temperature), at 32.5°C produces a male-biased sex ratio (approximately 75% male) (male-biased incubation temperature), and at 34°C again produces virtually all females (high-tem-

perature) ([79], Crews, unpublished data) (Fig. 1). While temperature determines the probability of developing as a male or female, temperature and sex effects are still separable at the two intermediate temperatures. For instance, comparisons between same-sex individuals from the male-biased and female-biased incubation temperatures elucidate temperature effects, while differences between males and females from the same incubation temperature elucidate sex effects. Temperature effects can also be analyzed across the full range of viable temperatures for females. Due to the fact that growth is contingent upon thermal regimens, especially for ectothermic vertebrates (see below), in all of the studies individuals are isolated and raised in as identical thermal environments and feeding regimens as possible.

We have found that incubation temperature affects growth rates and final adult size as well as the endocrine physiology, brain organization, aggressive and sexual behaviours and reproductive success of adult leopard geckos.

3. Morphology and growth

Both gonadal sex and incubation temperature affect the development of such morphological traits as pre-anal pores, hemipenes and head width. Males have open secretory pores anterior to the cloaca called pre-anal pores and paired intromittent organs called hemipenes. While preanal pores are closed in low-temperature females, they are open in females from the male-biased temperature [17]. In general, the development of these traits is plastic and androgen-dependent. Castration of adult males results in pore closure while short term (i.e. 30 days) androgen treatment of adult females induces limited development of these pores [58]. Conversely, males retain their hemipenes even after castration. Interestingly, long-term treatment androgen of adult females induces hemipenile growth (Rhen and Crews, unpublished data). Males also have wider heads than females. Nonetheless, incubation temperature also affects within-sex variation in this trait as females from a male-biased temperature have wider heads than low-temperature females [17]. This may be a result of higher androgen levels in individuals from the male-biased temperature (see below).

Research on rodent growth physiology suggest that sex steroids, thyroxin and/or glucocorticoids are responsible for sexual dimorphisms in body size. In general, it appears that estrogens inhibit, while androgens stimulate, growth in rats. Sexual dimorphisms, in body weight and longitudinal bone growth are conspicuous by 30 days of age, with males being larger [28]. This effect is mediated through such trophic factors as growth hormone and thyroxin. Neonatal administration of T leads to increased body weight, decreased

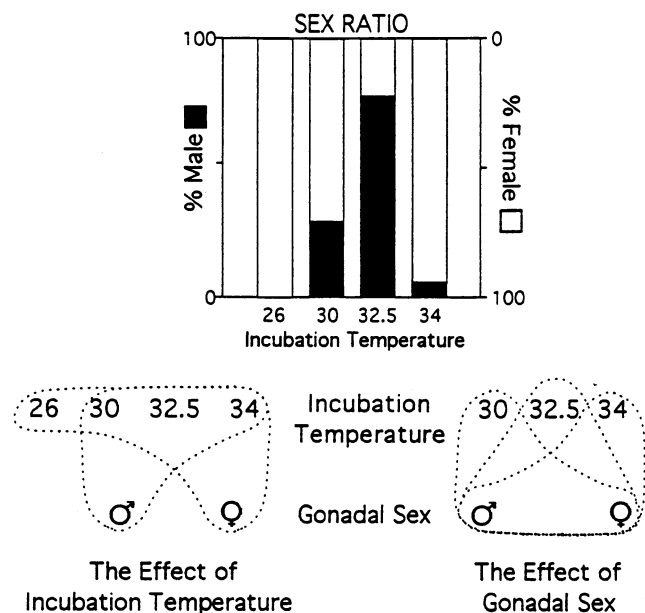


Fig. 1. Influence of incubation temperature on sex ratio in the leopard gecko (*E. macularius*) (top panel). The effects of temperature are revealed by studying same-sex animals that differ only in their incubation temperature (bottom left panel), whereas comparing males and females from the same incubation temperature reveals the effects of gonadal sex (bottom right panel). Dotted lines group the different comparisons. Modified from Coomber et al. [16].

Table 1
Incubation temperature modifies how female leopard geckos (*E. macularius*) respond to hormone manipulation

Incubation temperature (°C)	Manipulation	n	Mean	SE
26	SHAM	6	36.07	3.2
	OVX	7	51.50	4.0
	EB	6	35.03	4.4
32.5	SHAM	7	44.63	3.6
	OVX	3	42.10	0.7
	EB	10	45.83	1.2
Male (32.5)		10	49.12	2.0

Only females are produced at a 26°C whereas a male-biased sex ratio is produced at 32.5°C. Eggs received 10 µg of estradiol benzoate (EB) during the mid-trimester of development. Ovariectomy (OVX) or sham-operation (SHAM) occurred on the day of hatch. Presented is the average body weight of females at 65 weeks of age; weight of males at same age also presented for comparison. Data from Tousignant and Crews [77]

estrogen production and decreased responsiveness to the weight-depressing actions of ovarian hormones, whereas adrenal stress hormones (e.g. glucocorticoids) retard growth [40].

In the leopard gecko, body size (i.e. mass and snout-vent length) is also sexually dimorphic, with males being the larger sex. Again, incubation temperature determines much of the variation in growth. Females from the male-biased temperature grow faster and larger than do females from a female-biased temperature [78] (Table 1). Males show a similar pattern of temperature effects on body size; males from the male-biased temperature are larger than males from the female-biased temperature.

Thyroxin (T4) levels in adulthood are positively correlated with body size (Crews et al., unpublished data). For example, at the male-biased temperature, males are the larger sex and have significantly higher T4 levels. Interestingly, adult female leopard geckos have higher corticosterone levels than males [78], which suggests that glucocorticoids may suppress female growth. Thus, at least some of this variation may be attributable to temperature-induced differences in growth physiology.

The interaction of the environment and the nervous and endocrine systems is especially evident when we consider that adult females from the female-biased temperature have higher plasma E2 levels compared to adult females from the male-biased temperature [78], suggesting a growth-inhibiting role of estrogen. Further, females ovariectomized at hatching from the low incubation temperature grow larger and faster than sham-operated females from the same temperature [78]. Specifically, at 65 weeks of age (i.e. adulthood), females ovariectomized at hatching are heavier and longer and have wider heads than unmanipulated females (Table 1). On the other hand, ovariectomy does not significantly affect growth rates of females incubated at the

male-biased temperature. Since only low-temperature females exhibit enhanced growth following ovariectomy, it appears that incubation temperature influences the growth-inhibiting effect of ovarian steroids during juvenile development. Nonetheless, sex steroids administered during embryonic development do not appear to influence subsequent growth; females hatched from oestrogen-treated eggs have similar growth profiles to females hatched from untreated eggs from the same temperature. Similar experiments on males and females from other temperatures have yet to be conducted.

The laboratory has collected data on the weight and snout-vent length (SVL) of adult females from birth. Both body weight and SVL increase over time in all females, and the rates of increase in both parameters is comparable across incubation temperatures (Sakata and Crews, unpublished data). Females from the male-biased incubation temperature are consistently significantly larger than females from the low and high incubation temperatures, and females from the male-biased and high incubation temperatures are consistently significantly heavier than females from the low incubation temperature [78]. Weight increases at a faster rate than SVL in adulthood, as revealed by an increase in the ratio of weight over SVL over time. However, females from different incubation temperatures invest energy differentially into the parameters. Females from the male-biased and high incubation temperatures have significantly higher weight:SVL ratios than females from the low temperature, suggesting that less energy is dedicated to increases in body size in females from the two higher incubation temperatures (Sakata and Crews, unpublished data). This trend is especially evident in females from the high incubation temperature, which have the highest weight:SVL ratio. Females from the female-biased temperature have intermediate values for all three parameters.

4. Reproduction and sociosexual behaviour

Incubation temperature also has long-term effects on reproductive physiology (Fig. 2). The androgen levels in neonatal and adult males are, respectively, roughly 10 and 60 times higher than in neonatal and adult females (Table 2) [34,78]. Moreover, incubation temperature affects within-sex differences in steroid levels ([34,78]; Crews, unpublished data). Oestrogen levels are higher in males from female-biased and high incubation temperatures compared to males from a male-biased temperature. Among females, circulating oestrogen levels are higher in females from the female-biased temperature compared to females from the low and male-biased temperature [78]. Additionally, androgen levels are lower in low-temperature females than females from the male-biased temperature. The ratio of total androgens

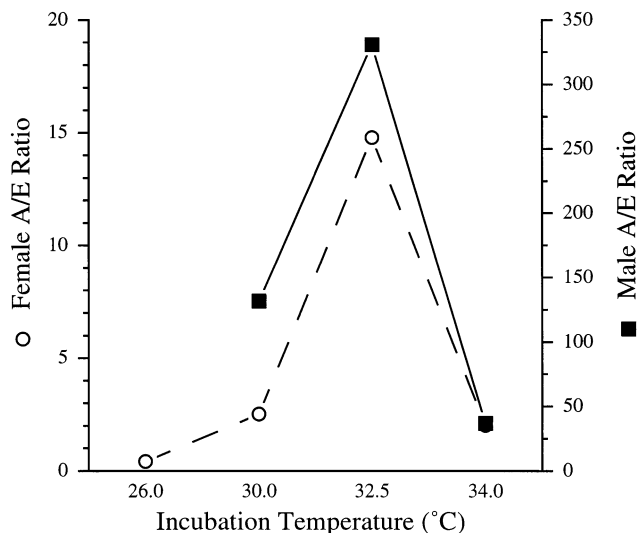


Fig. 2. Ratio of the plasma levels of total androgens (A) and estrogens (E) in adult female (dashed line) and male (solid line) leopard geckos (*E. macularius*) from different incubation temperatures. Data from Coomber et al. [16] and Gutzke and Crews [34].

to oestrogens fluctuates across temperatures in both males and females in a manner that parallels sex ratio (Fig. 2). The organizational mechanism underlying these intrasexual and intersexual differences in plasma levels of sex steroid hormones is unknown. However, one piece of evidence points to direct temperature effects on endocrine phenotype. Androgen and oestrogen levels in females treated with E2 during embryonic development were not different from circulating levels in untreated females that had been incubated at the same male-biased temperature [60].

Behavioural aggression also varies between the sexes and across incubation temperatures in a manner that parallels sex ratio. In general, males display offensive aggression and will posture and attack another male as he approaches. Females, on the other hand, exhibit defensive aggression; i.e. females show little or no aggression in response to females or males unless first

Table 2

Circulating concentrations of steroid hormones in hatchling and adult leopard geckos from a female-biased incubation temperature (30°C)

Sex	Number	Testosterone	Estradiol-17 β
Hatchling			
Male	5	1.43 (0.70)	0.14 (0.03)
Female	6	0.20 (0.02)	0.12 (0.02)
Adult			
Male	6	77.92 (26.40)	0.48 (0.6)
Female	7	1.23 (0.45)	0.49 (0.05)

Mean concentration (ng ml⁻¹) is presented; standard error in parentheses. Due to the small blood volumes of hatchlings, samples were pooled ($n = 2-6$ hatchlings/pool); number indicates number of pooled blood samples. For adults, number indicates individual animals.

attacked. Although males rarely attack females, males from a female-biased temperature are less aggressive towards females than males from the male-biased temperature [27]. Similarly, females from the low and female-biased incubation temperatures are less aggressive than females from the male-biased and high incubation temperatures.

Aggressive behaviours can be induced in both males and females by treatment with exogenous T. However, incubation temperature influences sensitivity to T treatment in adulthood within females. Following ovariectomy and T treatment during adulthood, low-temperature females do not exhibit increased aggression towards male stimulus animals, whereas females from the male-biased temperature return to their high preoperative levels [26]. The effect of ovariectomy also appears to be organized by incubation temperature; following ovariectomy and treatment with cholesterol during adulthood, the percentage of females from the low temperatures responding aggressively to male stimulus animals increases while that for females from the male-biased temperature decreases. Aggressiveness is readily reinstated upon T administration in males.

Courtship is a male-typical, androgen-dependent behaviour. In a sexual encounter, the male slowly approaches the female, first licking the substrate or the air with his tongue and then licking the female. An attractivity pheromone contained in the skin of females [50] elicits a male-typical tail-vibration that creates an audible buzz and a tactile vibration in the substrate. Tail-vibration increases the likelihood that a female will display receptive behaviour (Rhen and Crews, unpublished data). Intact males from the female-biased incubation temperature display more tail-vibrations during 5-min behavior tests with female stimulus animals [27]. Intact females have never been observed to exhibit tail-vibrations, regardless of their incubation temperature. Although T-treatment of ovariectomized females will cause them to exhibit tail-vibrations toward female stimulus animals [26], the total frequency of these tail-vibrations is much less than in similarly treated males. While castrated males exhibit courtship behaviours after treatment with E2 or dihydrotestosterone (DHT), ovariectomized females from these same incubation temperatures will not show such behaviours even with identical hormone treatments (Rhen and Crews, unpublished data). In summary, the sexes are organized differently in terms of their sensitivity to hormone treatments: T and its metabolites will activate courtship behaviours in the male, whereas only T activates courtship in females.

Following tail vibrations, males grip a female's skin with their jaws during their mounting attempts. Interestingly, the intensity of this behaviour is affected by a male's embryonic incubation temperature: males from the female-biased temperature body grip for a longer

duration than males from the male-biased temperature during behaviour tests with a receptive female. There are also sex differences in the activation of body grips; DHT and T activate this behaviour in males whereas females do not display this behaviour even when treated with androgen implants that produce male-typical hormone levels (Rhen and Crews, unpublished data).

Attractivity also varies with circulating hormone levels, gonadal sex and incubation temperature. Attractiveness is a female-typical trait and is measured by the intensity and frequency of a sexually active male's courtship behaviour toward the test individual. Intact females from the female-biased and low incubation temperatures are more attractive than females from the male-biased temperature. Furthermore, attractiveness is influenced by gonadal steroids: ovariectomized females treated with either cholesterol or E2 are more attractive than androgen-treated animals ([26]; Rhen and Crews, unpublished data). Although intact males are never attractive to other males, long-term castrates are courted by intact males.

Receptivity in leopard geckos is a female-typical behaviour that is characterized by a tail-lift that exposes the cloaca for intromission. Preliminary studies suggest that receptivity is organized by gonadal sex during development and activated by circulating hormones in adulthood [58]. Adult females display receptivity when treated with E2, but not when treated with cholesterol or androgen implants that produce male-typical hormone levels. Male castrates display very little receptive behaviour, even when treated with a hormone regimen that stimulates receptivity in females.

Differences in the proportion of infertile eggs that are laid by females from different incubation temperatures may be an indirect indicator of differences in receptivity to male courtship. High-temperature females tend to lay a higher proportion of infertile eggs than females from any other temperature (Sakata and Crews, unpublished data). Thus, the aggressive behaviour of these females towards males may have consequences for their reproductive success [26]. Alternatively, it may be that high-temperature females mate normally but that sperm are somehow unable to fertilize the egg. In contrast, females from the female-biased temperature lay the lowest proportion of infertile eggs. Furthermore, both low- and high-temperature females tend to lay eggs of lower viability than females from the intermediate incubation temperatures (i.e. a lower proportion of fertilized eggs survive until hatching). We have not detected any incubation temperature effects on male reproductive success in our colony.

5. Brain organization

As all behaviour is governed by the nervous system,

we have examined the effects of incubation temperature and gonadal sex on neural phenotype. Specifically, we have studied how the size and metabolic capacity of limbic and nonlimbic brain areas vary among individuals from the different incubation temperatures. Alternate sections were stained for Nissl substance so that the volume of identified brain areas (Fig. 3) could be measured. Changes in nucleus size reflect changes in the number of neurons, the size of neurons or both and are assumed to reflect functional changes. In addition, COX histochemistry was performed on other brain sections from the same animals. COX is an excellent marker of metabolic capacity because it is the rate-limiting enzyme in oxidative metabolism; increased activity of neurons in a brain region leads to increased COX activity in their mitochondria [82]. Studies of metabolic potential complement studies of nucleus size, yet COX may be a better assay of alterations in neural function (see below). For the purposes of this paper, the regions of interest are the POA and the ventromedial hypothalamus (VMH); both areas are critical for the expression of male-typical and female-typical sexual behaviours in many vertebrate [21,56,63].

In the leopard gecko, there are no significant differences in the volume of the POA or the VMH between males and females from the same incubation temperature (Fig. 4) [16]. There are, however, consistent differences across incubation temperatures, suggesting that nucleus size is regulated primarily by incubation temperature. The volume of the POA is larger in both males and females from the male-biased temperature compared to same sex animals from the female-biased temperature. Conversely, the volume of the VMH is larger in low-temperature females compared to females from the male-biased temperature. In other words, the volume of brain areas seem directly related to incubation temperature, not circulating levels of sex hormones.

We have found that in the leopard gecko, T treatment elicits male-typical courtship behaviours in ovariectomized females from low and male-biased temperatures (Fig. 5). While parallel metabolic changes in the POA are observed in ovariectomized females from both temperatures, significant structural changes are found only in females from the male-biased temperature. In other words, in females from the male-biased temperature, both COX and volume changes in the POA parallel changes in the frequency of courtship behaviours, while in low-temperature females, only metabolic capacity in the POA shows significant changes that parallel behaviour. Thus, it appears that changes in COX expression in the POA correlate more with the expression of courtship behaviors than do changes in POA volume.

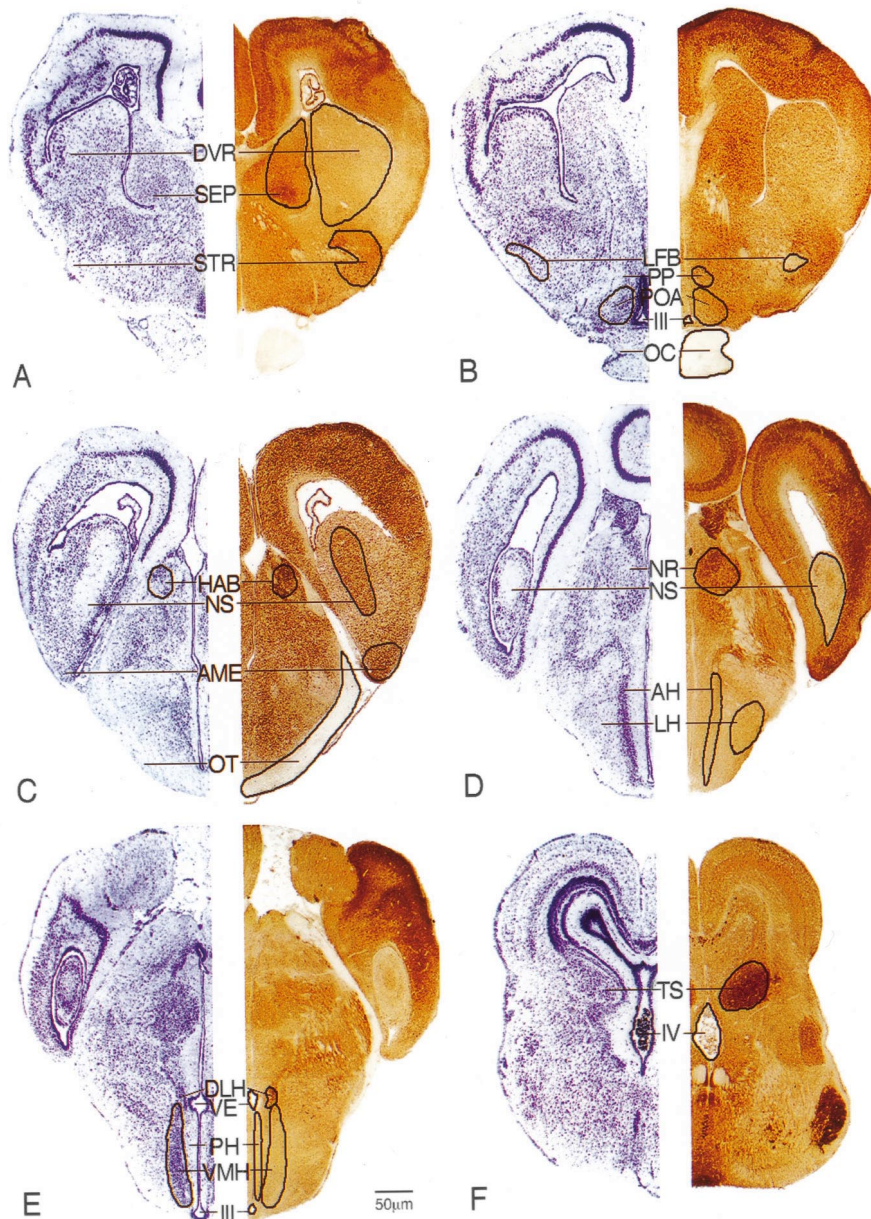


Fig. 3. Adjacent sections from the leopard gecko (*E. macularius*) stained with cresyl violet (left) or histochemically stained for cytochrome oxidase (C.O.) activity (right); coronal sections with dorsal at the top and medial to the center of each picture. The brain depicted is from a 1-year-old male from an egg incubated at a male-biased temperature. A–F represent a rostral-caudal series of sections. Boundaries of the brain areas measured are traced. In left panels the areas measured for volumetrics are traced, whereas in the right panels the areas measured for C.O. activity are traced. Bar = 50 μ m. Photographs digitally processed. AH, anterior hypothalamus; AME, external amygdala; DLH, dorsal lateral nucleus of the hypothalamus; DL, dorsal lateral nucleus of the thalamus; DVR, dorsal ventricular ridge; HAB, habenula; LFB, lateral forebrain bundle; LH, lateral hypothalamus; NR, nucleus rotundus; NS, nucleus sphericus; O.C., optic chiasm; OT, optic tract; PH, periventricular nucleus of the hypothalamus; POA, preoptic area; PP, periventricular nucleus of the preoptic area; SEP, septum; STR, striatum; TS, torus semicircularis; VE, ventricular ependymal organ; VMH, ventromedial nucleus of the hypothalamus; III, third ventricle; IV, fourth ventricle.

Incubation temperatures and gonadal sex interact to influence the metabolic capacity of hypothalamic nuclei in the leopard gecko. Males generally have greater COX activity in the POA, whereas females have greater COX activity in the VMH [16] (Fig. 4). These sex differences in COX activity in the POA are not found among individuals from the male-biased temperature

but are present at the female-biased and high incubation temperatures. In addition, males from the female-biased incubation temperature have greater COX activity in the POA relative to males from the male-biased incubation temperature and, as noted above, males from the female-biased incubation temperature exhibit higher levels of courtship behaviors (i.e. tail

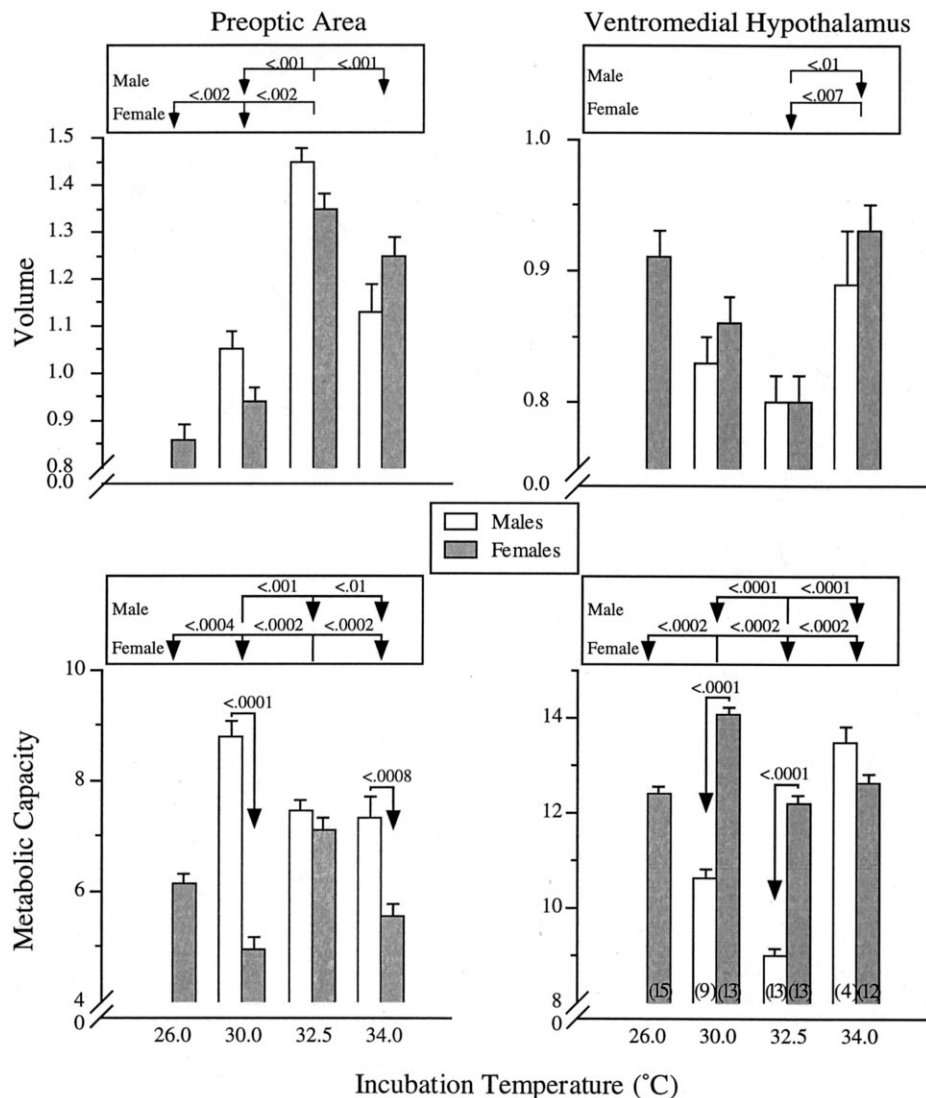


Fig. 4. Effect of incubation temperature and gonadal sex on the volume (top panels) and COX activity ($\mu\text{mol min}^{-1}$ per g tissue wet weight) (bottom panels) of the POA (left panels) and VMH (right panels) in the leopard gecko (*E. macularius*). Volumes are normalized by entire forebrain volume. Significant differences (entries are *P* values) within each sex are illustrated in box above each panel, indicating the effect of incubation temperature. Significant differences between the sexes are illustrated above bars, indicating the effect of gonadal sex. Sample sizes in parentheses. Means depicted with vertical bars representing standard error. Data from Coomber et al. [16].

vibrations) than do males from the male-biased incubation temperature. Further, females from the female-biased incubation temperature have greater COX activity in the VMH compared to females from all other incubation temperatures. The VMH plays a critical role in the expression of female receptive behaviors, and the finding that females from the female-biased incubation temperature have the highest COX levels in the VMH correlates with their high levels of receptive (as inferred by egg fertility data; see above).

6. Neuroendocrine mechanisms underlying incubation temperature and sex effects on growth and reproduction

Although incubation temperature and gonadal sex during development appear to organize growth and reproduction, the neuroendocrine mechanism(s) underlying such effects are unknown. Three hypotheses may explain the relationship between sex ratio and masculine phenotypes. One hypothesis is that a single underlying mechanism (i.e. sex steroids) has pleiotropic effects on sex determination, the brain and behaviour.

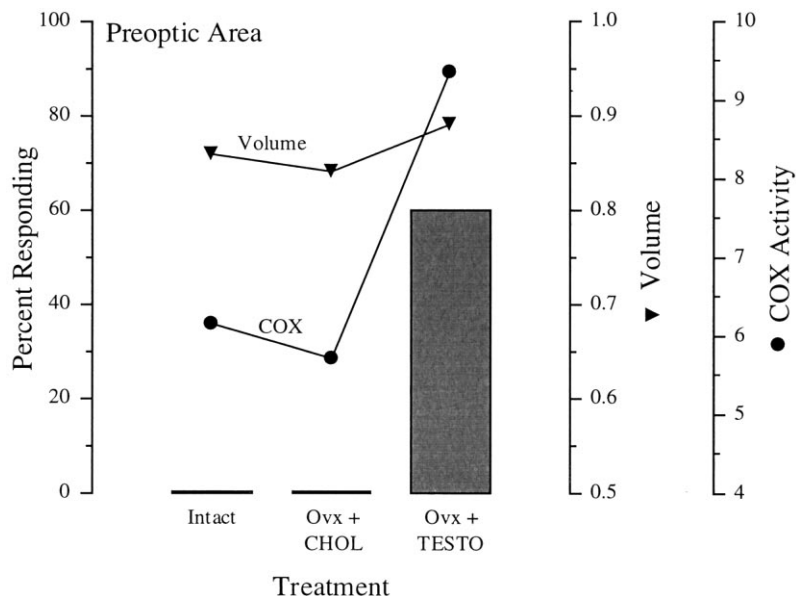


Fig. 5. The effects of gonadectomy and treatment with exogenous testosterone or cholesterol in female leopard geckos (*E. macularius*) from a low incubation temperature on the volume and metabolic capacity (COX activity) in the POA and frequency of male-typical courtship in response to female stimulus animals. Note that the correlation between behavior and metabolic capacity is stronger than that between behavior and nuclei size. OVX, ovariectomy; CHOL, cholesterol; TESTO, testosterone. Data from Crews et al. [22].

This hypothesis is tenable because TSD in reptiles and behavioural organization in mammals both involve production of sex steroids during critical periods of development. In other TSD species, temperature induces production of sex steroid hormones which, in turn, cause gonadal differentiation in a threshold-dependent manner; that is, intersexes are rarely produced [20]. Not only does the diencephalon differentiate at the same time as the temperature-sensitive period of gonadal development [73], but new evidence suggests that the nervous system may be involved in transducing the temperature signal into the physiological signal for gonadal differentiation; in the Olive Ridley sea turtle, there are higher levels of E2 in the embryonic diencephalon at a female-producing temperature than at a male-producing temperature, suggesting a temperature-sensing function within the developing nervous system [65]. If brain and behavior in leopard geckos are organized by sex steroids, this model of steroid production may explain why neural and behavioral phenotypes and sex ratio (i.e. percent male) are correlated as a function of incubation temperature: aggressiveness, androgen levels and POA volume and metabolic capacity are positively correlated with sex ratio whereas attractiveness, receptivity, plasma estrogen levels and VMH volume and metabolic capacity are negatively correlated with sex ratio. Although this hypothesis does not address sexual dimorphisms within an incubation temperature, the importance of sexual dimorphisms in the leopard gecko may be slight considering the few such instances in the brain relative to within-sex differences (Fig. 4).

A second hypothesis is that sex differences in adult behavior may be the result of sex differences in steroid production after sex determination. Indeed, plasma levels of T differ in hatchling males and females from a female-biased temperature (Table 2). In many mammals, birds and lizards, perinatal sex steroid hormone secretions regulate sexual differentiation [38]. Thus, plasma levels of sex steroids may differ both between the sexes and among leopard geckos from different incubation temperatures.

While the first hypothesis postulates a transduction of incubation temperature into a physiological signal (i.e. indirect temperature effects through sex steroids), a third hypothesis is that incubation temperature directly organizes the brain. Preliminary evidence supports such a hypothesis for aggression and growth in females. Females hatched from estrogen-manipulated eggs incubated at the male-biased temperature do not differ in growth rates or aggressiveness from unmanipulated females from the same incubation temperature. Temperature also seems to directly affect neuroanatomy, as the volumes of the POA and VMH of males and females from the same incubation temperature are not significantly different while differences across temperatures are pronounced [16].

These three hypotheses, however, are not mutually exclusive. Indeed, there is some evidence for subtle interactions between embryonic temperature and later exposure to sex steroid hormones. Gonadectomy does not affect growth rates in females from the male-biased temperature but results in increased growth in females from the low incubation temperature (Table 1). Clearly,

we need to understand not only how temperature (either directly or indirectly) and gonadal sex have their independent effects but also how they interact to regulate growth and reproduction. A likely mechanism is found in the rat and presumably the leopard gecko as well. Four populations of temperature- and steroid-sensitive neurons exist within the POA-anterior hypothalamus (POAH) continuum: temperature-sensitive but not steroid-sensitive, temperature- and steroid-sensitive, temperature-insensitive but steroid-sensitive and temperature- and steroid-insensitive neurons [75]. Thus, it is plausible that neuronal populations sensitive to both sex steroids and temperature regulate growth and reproduction in the leopard gecko. In light of this, it would be instructive to consider the relationship between growth, thermoregulation and reproduction in other reptiles.

7. Growth and thermoregulation

Growth in ectothermic vertebrate is contingent upon behavioural thermoregulation (see review in Ref. [39]). Temperature regulation is a homeostatic mechanism. While mammals utilize both physiological (e.g. piloerection and shivering) and behavioural means to maintain species-specific body temperatures, ectothermic vertebrates, including many lizards, regulate body temperature primarily via behaviour and do so very precisely (e.g. seeking shade or basking, gaping, amphibious behaviour) [39]. One of the most common behavioural traits measured, preferred temperatures, correlates with the optimal temperature for many tissue and cellular functions [23]. It has been found that in a number of reptiles, preferred body temperatures increase after feeding [31,45,57]. Such behaviours increase digestive efficiency, allow increased feeding frequencies and augment growth potential. Thus, it is clear that the study of growth in ectotherms requires a thorough analysis of thermoregulatory behaviours.

Incubation temperature can influence behavioural thermoregulation in at least two TSD species. Lang [46] found that crocodiles from higher incubation temperatures selected higher temperatures and correspondingly grew to larger sizes. In this study, however, the effects of gonadal sex and incubation temperature were confounded as higher temperatures produce mostly males. Studies on the leopard gecko and the snapping turtle that experimentally separated the confounding effects of incubation temperature and gonadal sex reveal that male-producing temperatures produce faster growth rates, regardless of gonadal sex ([77]; Rhen and Lang, unpublished data). There is some evidence this effect may be mediated by behavioural thermoregulation as individuals from male-producing temperatures spend a greater proportion of time in warmer aquatic environments [55,59].

Given this link between incubation temperature, growth and thermoregulation, we should consider the neural substrates underlying thermoregulation and reproduction. The POAH mediates behavioural and physiological thermoregulation [4,5]. Temperature-sensitive neurons within this region are found in both mammals and reptiles [10,36,61]. While this area was thought to be a master controller for thermoregulation, a more recent hypothesis suggests that the POA is one of a number of sensor-effector systems that mediate independent thermoregulatory responses [66,67]. Indeed, multiple brain and peripheral regions are involved in different thermoregulatory responses of lizards [25]. Nonetheless, these independent systems appear to be arranged hierarchically with higher systems (especially the POAH) facilitating or inhibiting those systems below [11,25,35,67]. Due to the significance of the POA in both reproduction and thermoregulation, integration of both systems may occur here.

The pivotal role of ambient temperature in activating seasonal reproductive behaviours is evident in at least one reptile, the red-sided garter snake. In this species, as in other species, lesions of the POA abolish male-typical courtship behaviour and disrupt thermoregulatory behaviour [19,29,30,44]. Circulating concentrations of androgens peak in the summer and prime the male for courtship the following spring when increasing ambient temperature triggers emergence from hibernacula and activates courtship behaviour [18]. Despite that, lesions in the POA disrupt both sexual and thermoregulatory behaviour and that sex steroid priming and temperature activation are required for male-typical sexual behaviour, it is unclear whether the same neurons (i.e. neurons sensitive to both temperature and sex steroid hormones) mediate both behaviours or whether separate groups of neurons (i.e. temperature or steroid sensitive neurons) interact to regulate both behaviours. Temperature has stimulatory effects on reproduction in other lizards, presumably through the same region of the brain. Photoperiod and temperature interact to stimulate follicular development in the eastern fence lizard [49], while an analogous interaction regulates testis development in male green anoles [48]. In both species, as in mammals, ovarian and testicular recrudescence are dependent upon increases in hypothalamic GnRH and pituitary gonadotropin secretion.

While this chapter has focused on ectothermic vertebrates, it is important to note that endotherms are also sensitive to temperature effects during development. For instance, cooling newborn rats to 18°C for 2 h delays the T surge that normally occurs about 2 h after birth [62]. Such experimental manipulation of temperature is biologically relevant because neonatal mammals are ectothermic. Hyperthermia shortly after birth decreases the expression of sexual

behaviours in adult female rats [43] and significantly depresses circulating levels of luteinizing hormone in adult ewes [68]. Ambient temperature also appears to affect adult reproduction in humans, but the mechanism underlying this effect is unknown [6]. Clearly, more work is needed to understand the mechanisms that mediate the effects of environmental temperature on growth and reproduction. This field of research is especially important since the neuroendocrine control mechanisms are likely to be conserved in all vertebrates.

8. Evolutionary issues

Sexual dimorphism in size is prevalent in a number of vertebrate taxa. In species in which males are the larger sex, intrasexual competition over access to resources and, ultimately, females, has been argued as a driving force. Although the hypothesis that size predicts male dominance and territory ownership has yet to be directly tested in the leopard gecko, it is plausible that the observation that size correlates positively with sex ratio (i.e. percent male) reflects a mechanism underlying the evolution and maintenance of TSD in leopard geckos. Males from the male-biased incubation temperature tend to be larger and more aggressive than males from the female-biased incubation temperature, while males from the female-biased incubation temperature tend to court more. This suggests that differences in mating strategies exist between males. It would be interesting to test the hypothesis that males with more aggressive, territorial strategies have a higher reproductive success than males with other strategies. If this were true, this could be the mechanism underlying the maintenance, even the evolution, of TSD.

Size effects on fecundity in females have been found in many species of fish and reptiles. Most of these species have variable clutch sizes, whereas in the leopard gecko clutch size is fixed at two eggs; hence, the benefits of size may be relatively small for females. We have yet to discover any beneficial effects of size and incubation temperature on such measurements as age at maturity, the number of clutches per season or interval between clutches. Thus, the selective advantage of size differences are not immediately salient or may be a neutral result of selection pressure on sex ratio and male body size.

As noted above, male leopard geckos have wider heads and greater jaw musculature than females. It is possible that androgens underlie these sexual dimorphisms. Due to the fact that males exhibit high levels of aggression toward each other, intrasexual competition may have favored greater jaw musculature and increased body size in males. Alternatively, this dimorphism may have evolved to facilitate sex differences in niche utilization so as to minimize resource competition as has been suggested in birds, lizards and snakes [74]. As dominance

among females probably has little influence on female reproductive success, it is likely that increased head width in females from the male-biased incubation temperature is a neutral trait resulting from higher androgens during development or in adulthood.

Thus, in the leopard gecko suites of traits correlate with sex ratio as a function of incubation temperature. We suggest that the integrated nature of these environmental effects probably reflects both a mechanistic and functional link between growth and reproduction. The mechanistic basis of these correlated effects remain to be determined, but it is possible that the POAH is involved as it is intimately involved in growth, thermoregulation and reproduction.

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