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Research Article
Ecophysiological Relationships of Environment and Reproduction in Males of Rock Mouse (*Peromyscus difficilis felipensis*) at a Temperate Forest

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Abstract

Background and Objective: The ecophysiological relationship between reproductive parameters and environmental variables is mostly unknown in wild temperate species of small rodents, inhabiting mid-latitude, temperate forests. Here the goal was the relationship between testicular function, testes and body weight to seasonal variations of daylight length and temperature in adult males of the Rock Mouse (*Peromyscus difficilis felipensis*), in a two-year study. **Materials and Methods:** Spermatogenesis, spermatogenic function, content and production of testosterone and body and testicular weight were monthly evaluated in free-living *P. d. felipensis*, using standard, histological and biochemical (ELISA) techniques. **Results:** Although the presence of sperm in both testes and epididymis was found all year round, there was a seasonal pattern with a spring peak in testis volume and testosterone content and production; though, the testicular function was maintained until fall and winter. Conversely, bodyweight decreased during spring-summer, while it increased in fall-winter. There was an ecophysiological positive correlation of reproductive parameters with environmental gathered data, especially with daylight length (i.e., increased correlation during spring, $p<0.001$) and there was a slight or no participation of environmental temperature. **Conclusion:** In addition to daylight length, the seasonal pattern of testicular function in these temperate populations of mice, indicates that other environmental factors, waiting to be elucidated (e.g., phytochemicals intake through food), also contribute to its regulation.

Key words: *Peromyscus*, spermatogenesis, testicular function, temperate forest, daylight length, male androgens, testosterone

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Free-living mammals and especially rodents usually have one or two annual reproductive seasons, showing hormonal periodical cyclic variation that correlates to gamete production and displays of specific copulatory behavior patterns[1,2]. Androgens, such as testosterone, promote several reproductive aspects, including male mating behavior[3]. In turn, specific environmental conditions of the habitat, such as temperature, daily light hours, food availability, pluvial regime and climate shifts affect these aspects[4-8].

Testis volume, usually considered an indicator of sperm production in micro-mammals, is credited for both the individual sex performance and its potential reproductive success[9]. Testosterone plays a relevant role in testes growth, regulation of spermatogenetic division in meiosis and maintenance of spermatogenesis[10,11]. Testosterone production with its intra-gonadal and circulating concentration influences more general reproductive events; e.g., sex differentiation, mate selection and male behavior, together with reproductive success[12-14].

Data on rodent testosterone contents, testis morphology, behavior and reproductive outcome correlations usually come from high latitudes species, especially from laboratory rodents, or from wild species raised under controlled environmental conditions[6,15-19]. Moreover, documentation of external somatic characteristics in wild free-living individuals is often associated with the time of year and used as an indicator of the population's reproductive status. In captured micromammals, these characteristics usually are i) amount of gestating or lactating females and ii) testis size and position in males[20-22]. While this information may describe the reproductive biology of the species at an ecological level, it is also necessary to understand fundamental phenomena that regulate physiological and endocrine processes in free-living rodents. To contribute to ecophysiological knowledge from free-living rodents inhabiting midlatitude, temperate forests, we have been studying the reproductive biology and ecology (e.g., population ecology, diet, use of space) of two species of deer mice. One of them, a subspecies of the Rock Mouse (*Peromyscus difficilis felipensis*), is widely distributed on the wooded areas around Mexico City and those nearby in the State of Mexico, including coniferous and mixed forests[23,24]. In our study locations at the mountain ranges in western Mexico City, the reproductive activity of *P. d. felipensis* happens all year round but with two distinctive peaks, occurring during the spring and trough fall-winter, respectively[25]. The study aimed to examine whether there is a relationship between seasonal variations of two environmental conditions (daylight length, temperature) with testicular function (testis volume, spermatogenesis, production of testosterone and its intra-gonadal content) in male adults of *P. d. felipensis* inhabiting on two locations of these mid-latitude, temperate forests, along two years.

MATERIALS AND METHODS

Study areas: Cumbres del Ajusco National Park (CANP) is located in the southwestern part of Mexico Valley, within the Ajusco-Chichinautzin Mountain Range. Its climate belongs to the subhumid temperate type C(w1)(w)(b')i'[26]. Higher temperatures range from April-September (mean±standard deviation, 15±2°C) and lower temperatures through November-February (11±1°C). Rainfalls occur along May-October (152±22 mm), while the dry season follows in November-April (16±11 mm). Vegetation corresponds to a stratified temperate mixed-forest with seasonal plants and tiller grasses. Oyamel trees (*Abies religiosa*) and oaks (*Quercus* sp.) dominate the canopy of mixed plots of other conifers and broad-leaved trees. The understory includes abundant bushes, mainly *Senecio* and *Salix,* together with seasonal, herbaceous asteraceae, rosaceae and solanaceae, among others and tillers of hard tall grasses (*Muhlenbergia* sp., *Festuca* sp.) or zacatonales. The ground includes mosses, lichens and several fungi. Sampling location was at 19°13'37" N, 99°15'37" W and 3180 m.

Desierto de los Leones National Park (DLNP), it is located at the western slope of Mexico Valley at Las Cruces Mountain Range, a prolongation of the Ajusco Mountain Range. Its climate belongs to the most subhumid temperate type C(w2)(w')(b')ig[26]. Higher temperatures occur in April-July (12.6±6°C), while lower temperatures are December-February (8.1±2°C). The rainy season is on May-August (235±30 mm) and the dry season is December-February (12±4 mm). Vegetation at DLNP is mostly as in CANP, but with a predominance of oyamel forests at higher elevations. Sampling location was at 19°18'17"N, 99°19'14"W and 2180-3200 m.

Capture and preparation of mice: We collected 43 adult males of *P. difficilis felipensis*, along two years (January, 2013 to December 2014), in aforementioned sampling locations. Mice were monthly captured, using Sherman traps (H.B. Sherman Traps Co, Tallahassee, FL, USA), baited with oat flakes, then transferred to the Laboratory of Mammals at the Division of Biological and Health Sciences (DBHS) and killed by cervical dislocation. All animal manipulations were made according to international standards[27] and were approved by the Ethics Commission at DCBS, UAMI. We took standard measures (mm) and weight (g), before preparing corpses as skeleton voucher specimens[20] to house them at UAMI

Mammal Collection. Testes were surgically exposed and excised to measure its width and length conventionally (to the nearest 0.01 mm) and to calculate their volume using the geometric formula for a prolate spheroid[28]. Recognition of adult males followed Hoffmeister[29], together with a typical pattern of pelage color for the species[30].

Testosterone production: This and the next two following procedures were developed at the Laboratories of Animal Ecophysiology and Organic Synthesis, DBHS. We followed Salame-Méndez *et al.*[31,32] with some modifications. Right testicle gonadal tissue was introduced in Eppendorf tubes containing Krebs-Ringer buffer with glucose (KRBG, pH 7) without metabolic cofactors and 10,000 cpm of tritiated cholesterol (1,2-^{3}H specific activity-sa-47.7 Ci/mmol) ($C_{27}{}^3H$) (Nuclear New England, Boston, MA). Experimental tubes (with tissues) and control tubes (without tissues), were incubated 1 h at 36°C. Metabolic reactions were stopped by freezing; the tubes were then defrosted and tissues in the incubation dissolution were homogenized. Two aliquots were taken from each homogenate, one to evaluate the precursor's biotransformation ($C_{27}{}^3H$) into testosterone and the other to determine protein concentrations[33]. Testosterone extraction was made from diethyl ether by duplicate. The organic phase was separated from the aqueous phase in a dry-ice:acetone media and the organic phases were evaporated to dryness. Extraction efficiency was made by adding to five tubes, at random, ≈1,000 cpm tritiated testosterone (1,2,6,7,16,17-^{3}H) (sa 139 Ci/mmol) (Nuclear New England, Boston, MA); average extraction efficiency was 96.4±1.2% and results of each testosterone production were corrected, starting from percentage recovery. Each tube containing testosterone extracts was added diethyl ether:methanol (3:1, v/v) and samples were transferred to chromatoplates (20×20 cm, UV 250 nm radiation indicator, Merck). Testosterone extracts were separated by TLC, using (i) benzene:ethyl acetate (5:3, v/v) and (ii) benzene:methanol (90:10, v/v), as mobile phases. Ratio-to-front (Rf) reference testosterone corresponding areas, were visualized with a UV lamp. The silica was scraped and the androgen adsorbed to the silica was eluted with a mixture of diethyl ether:methanol (1:1, v/v); collected in glass vials and counted, after the addition of Instagel (Packard), in a liquid-scintillation spectrometer (Beckman, LS-7000) with maximum efficiency for tritium of 53%. Testosterone productions were expressed as a percentage of $C_{27}{}^3H$ biotransformation, related to 100 mg of gonad protein after 1 h incubation period.

Intra-gonadal testosterone content: Left testicle gonadal tissue was homogenized by sonication. The extraction of total sexual steroid contents in each homogenized was made by duplicated with diethyl ether. Evaluation of testosterone contents in gonadal tissue was made by enzyme-linked immunosorbent assay (ELISA), using commercial kits (DRG Instruments Inc.® Frauenberg, Marburg. Germany) and following instructions provided in manufacturer's manual. Testosterone concentration was determined from a spectrophotometer (Microplate Reader, MR 600, Dynatech Product® Dynatech, Chantilly, VA. USA). The specificity of testosterone antiserum and linearity described by the kit manufacturer was validated by testing the solutions of the hormone provided in the kit, as well as from solutions of testosterone (T) and rostenedione (A) and progesterone (P_4) (Sigma Labs, Santa Fe, NM, USA), previously purified. Percentage of cross-reactivity (specificity) was 100 for T; 0.8±1.23 for A and <0.1 for P_4 and some of those reported by the manufacturer: 100 for T; 6.6 for 5α-dihydrotestosterone; <0.1 for dehydroepiandrosterone; 0.9 for A and <0.1 for P_4. The sensitivity (minimum detectable concentration) of the assay for testosterone was 83 pg.

Spermatogenesis evaluation: Assessment of spermatogenesis was developed in the Laboratory of Animal Ecophysiology. A biopsy taken from each left testicle and both epididymis was then placed within a formalin-buffered solution and included in paraffin. Serial sections (3-5 μm) were processed for staining with hematoxylin-eosin. Spermatogenic and spermiogenic processes were determined following Fawcet[34]: Phase 1, or spermatocytogenesis, involves the development of spermatogonia until its differentiation into preleptotene spermatocytes; Phase 2 involves the meiotic process, taking place within spermatocytes, which differentiate into spermatids and Phase 3 or spermiogenesis, corresponds to the differentiation of spermatids into spermatozoids.

Environmental conditions: This and the next procedures were carried out at the Laboratory of Mammals, DBHS. Monthly average data for daylight length and temperature were obtained from the nearest meteorological station (Ecoguardas Ajusco (EAJ) 19.27°N, -99.20°W, 2584 m) and TUTIEMPO Network (https://www.tutiempo.net) during the study period.

Statistical analysis: Analysis of variance (ANOVA), followed by Tukey's multiple comparisons, were used to examine average behavior of testicular function (testicular volume, testosterone production, intra-gonadal testosterone content) and individual weight by age, location, month and season of the year (data pooled in trimesters; e.g., March-May into spring and so forth). To establish monthly and seasonal behavior of individual weight, testicular volume variation was eliminated

through a covariance analysis (ANCOVA). Linear correlations between individual weight, testis volume, testosterone production and testosterone intragonadal content were analyzed through simple linear regressions. Whereas dependence of testicular function (testis volume, testosterone production and testosterone intra-gonadal content) to environmental conditions (daylight length and temperature) were obtained through a Pearson's multiple comparison matrix. All analysis were performed with the statistical packages GraphPrisma® [35] and NCSS[36], under a significance level of $\alpha<0.05$.

RESULTS

Figure 1a shows monthly testicular function in adult males of *Peromyscus difficilis felipensis*. Testosterone

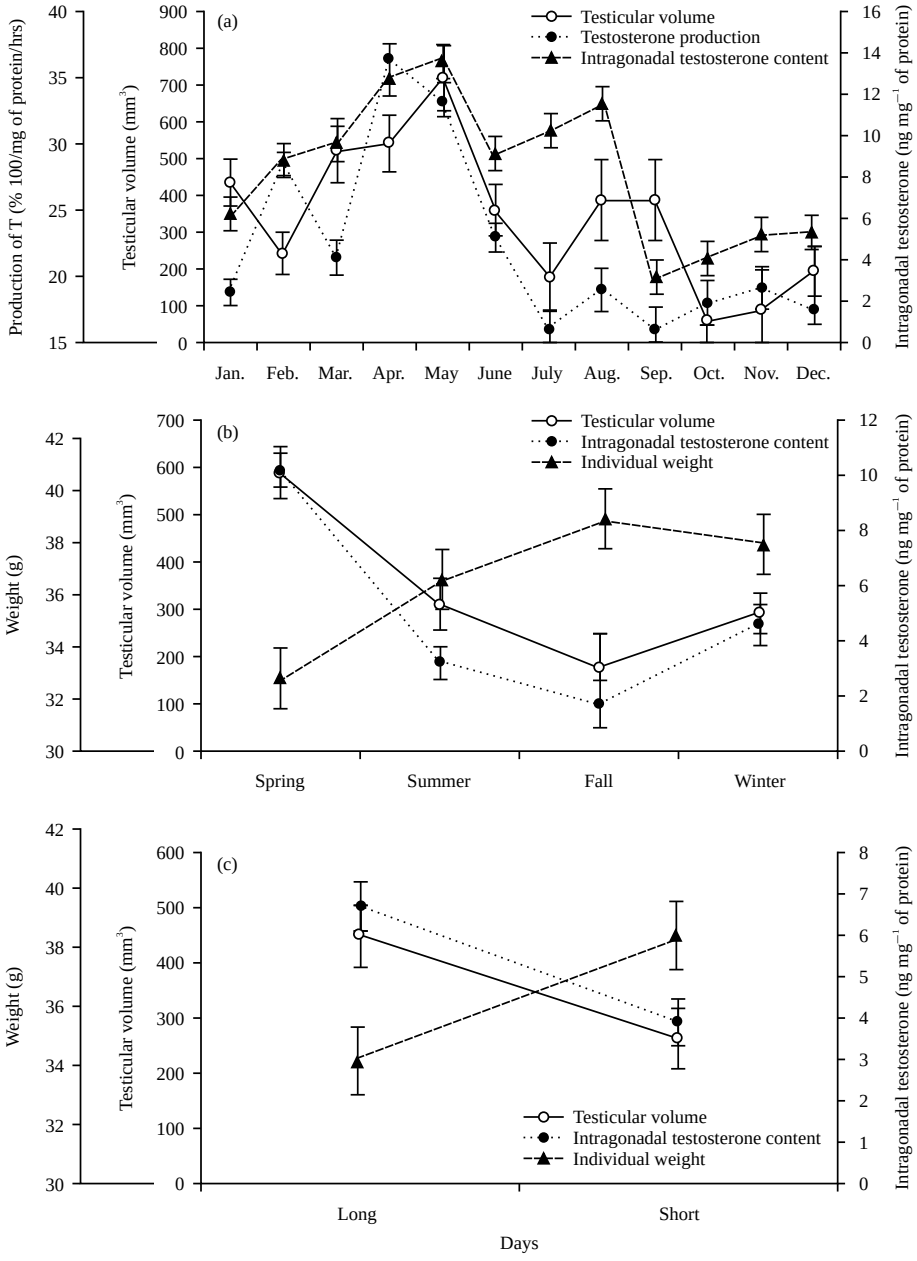

Fig. 1(a-c): Temporal variations of testicular function (testicular volume, testosterone production and intragonadal testosterone content) and individual weight in adult males of *Peromyscus difficilis felipensis*, together with their dependence on daylight length: (a) Monthly variation of testicular function, (b) Seasonal variation of testicular function and (c) Dependent patterns of testicular function, according to daylight length

Table 1: Seasonal means±standard deviations for somatic and physiological variables in adult males of *Peromyscus difficilis felipensis* and for environmental variables

Parameters	Spring (n = 10)	Summer (n = 10)	Autumn (n = 5)	Winter (n = 18)
Body weight (g)	37.40±5.10	35.77±19.3	35.20±4.33	37.00±6.49
Testis volume (mm^3)	588.64±70.31*	310.24±5.77*	120.92±15.99*	292.36±179.03*
Testosterone production (biotransformed precursor %)	33.24±2.94*	29.63±2.44*	22.55±1.72*	26.22±2.68*
Intragonadal testosterone content (ng/mg protein)	10.19±4.92*	3.24±2.32*	1.93±0.89*	4.61±3.42*
Temperature (°C)	18.40±1.71*	19.30±3.16*	16.00±1.22*	13.05±0.87*
Daylight Length (h. min±min)	12.33±25*	13.9±12*	11.40±26*	11.20±22*

*: Significant statistical differences, $\alpha \leq 0.05$

production (Fig. 1a) was higher in April, May and August ($F = 24.54$, $df = 11$, $p<0.0001$), whereas its lowest levels were found from September through November ($F = 24.54$, $df = 11$, $p<0.0001$). Intra-gonadal testosterone content (Fig. 1a) starts at a basal low level in January (2.425±0.614), increases through February-March to reach a peak by April-May (13.653±0.752, $F = 4.93$, $df = 11$, $p = 0.00019$) and decreases from June to December, where basal levels are maintained at almost constant values (3.25±1.37) for all three seasons. Testis volume (Fig. 1a) was statistically higher during May ($F = 30.82$, $df = 11$, $p<0.0001$) than in every other month, even when slight variations were found all year long ($F = 1.51$, $df = 11$, $p = 0.1772$); then, from June through February, testis volume was statistically lower ($F = 30.82$, $df = 11$, $p<0.0001$).

Table 1 and Fig. 1b show seasonal changes of testicular function and weight, the former also shows seasonal changes in daylight length and temperature. Testosterone production (intra-gonadal testosterone contents, $F = 10.42$, $df = 3$, $p<0.0001$) and testis volume ($F = 8.89$, $df = 3$, $p = 0.0001$) were significantly higher during spring. A significant decrease for testosterone production was noted from summer through fall ($F = 24.54$, $df = 11$, $p<0.0001$) but with an increase by winter (Fig. 1b). Intra-gonadal testosterone contents was significantly lower in fall and mostly variable during summer or winter ($F = 4.93$, $df = 11$, $p = 0.0002$). Although no significant variations in body weight were recorded along the year ($F = 2.64$, $df = 3$, $p = 0.0631$, Table 1), it was higher during the shorter day seasons (fall-winter, Fig. 1b,c).

Pearson's correlation analysis showed no correlation between daylight length and testis volume ($r = 0.28$, $p = 0.06$), whereas daylight length and environmental temperature (Fig. 1c) had a significant positive correlation with the synthesis of testosterone ($r = 0.57$, $p<0.0001$) and with intra-gonadal content of the androgen ($r = 0.32$, $p = 0.03$). In fact, dependence was higher during larger daylight length through spring-summer, than during shorter daylight length in fall-winter (testosterone production: $F = 41.21$, $df = 1$, $p<0.0001$; intragonadal testosterone content: $F = 9.40$, $df = 1$, $p = 0.0037$; testis volume: $F = 4.85$, $df = 1$, $p = 0.0331$). Conversely, individual weight was significantly lower during longer daylight in spring-summer ($F = 41.21$, $df = 1$, $p<0.0001$; Fig. 1c).

Spermatogenesis in *Peromyscus difficilis felipensis* was as follows. Spermiogenic processes (Phase 3) were recorded from testicular tubules in adults captured in January, whereas spermatocyte to preleptotenic spermatid differentiation (Phase 2) was the predominant phase in the germinative epithelia in testes from individuals captured during February, with an alternating predominant pattern. We also observed abundant mature spermatozoa in epididymis from animals captured all year long. Therefore, when taken together, these data indicate that the spermatogenic cycle lasts 34±4 days in *P. difficilis felipensis*.

DISCUSSION

Although spermatogenesis occurred all year round in males of *Peromyscus difficilis felipensis*, evidences showed a seasonal pattern with a spring peak for other testicular functions and body weight. Adult males of *Peromyscus difficilis felipensis* had sperm in both testicles and epididymis throughout the year, indicating a continuous active and enduring gamete production (34±4 days), which is consistent with the continuous production and intra-gonadal content of testosterone, the androgen that regulates meiosis during spermatogenesis and maintains spermiogenesis[11]. On the other hand, together with higher spring values for testis volume, testosterone production and intra-gonadal testosterone content indicate that this is the preferred reproductive season for *P. d. felipensis*, especially from April to May (Table 1). Supporting this fact are the largest numbers of pregnant and/or lactating females also recorded during late spring and in summer[25]. This supports that favoring of reproductive activity by *P. difficilis* in spring is related to days with longer light hours that precede and occur in the summer rainy season and, thus, promote a more diverse understory (i.e., more food availability) in both National Parks.

On the other hand, one verified fact was that position of testes was not always associated to reproductive activity of males of *P. d. felipensis*. In some mice with abdominal testes, both production and intra-gonadal content of testosterone were similar to those in mice with scrotal testes; the former also presented spermatogenesis in phases 2 and 3, as well as

gametes in the epididymis all year round. Other cricetids studied under either natural or controlled conditions show similar evidence. We found the same pattern in free-living populations of the congeneric syntopic species *Peromyscus melanotis*[25,28,37] in our study areas, while Boiani *et al*.[38] reports this fact in *Oligoryzomys flavescens* from a temperate boreal habitat. Moreover, in a laboratory colony of *Neotomodon alstoni*, Olivera *et al*.[39] found reproductively active males, even though they had no scrotal testicles. Therefore, inferring reproductive activity just from the sole observation of scrotal testes might prove to be inaccurate for some species of wild micromammals.

There was a positive dependence of the reproductive physiological variables to daylight length. Even though the correlations between daylight length with the production of testosterone and its intra-gonadal content were both low in *P. d. felipensis*, there is a positive dependence of the physiological variables to this abiotic one as noted elsewhere[40]. This is reinforced by the fact that testes of mice from the seasons with longer light hours per day (spring-summer), showed significantly higher synthesis and contents of testosterone, as compared to mice from shorter daylights in fall-winter. Therefore, we can suggest that there is a positive dependence between both environmental conditions, but especially with daylight length (Fig. 1c) and testicular function (testosterone production and intra-gonadal testosterone content). Taken together, our results agree with some studies of wild rodents under laboratory conditions, where circulating testosterone concentration increased or decreased in response to light time[7,41-43].

On the other hand, the correlation between testicular volume and both testosterone production and intra-testicular content, suggests that testicular function could be rather stimulated by both abiotic (e.g., light hours) and biotic environmental factors (e.g., type of food), which interact with the species genetic reproductive background. That is, although the highest reproductive peak for this species occurred during spring, reproductive activity was recorded until summer, as well as in fall-winter, even though testosterone production, intra-testicular testosterone content and testis size decreased. Moreover, our records of spermatozoa in the epididymis, agree with our results from a previous study[25]: these indicators for testicular function also went down but did not cease through apparently less favorable conditions for the reproductive activity, such as the colder and dryer seasons of autumn and winter. Also, seasonal dependence between spermatogenesis and testicular volume with testosterone production and intra-testicular testosterone content, allowed us to verify that these indicators increase during the seasons with longer light hours (spring-summer), while they diminish, though without ceasing, during those days with shorter light hours (fall-winter).

The mild hardening of environmental conditions of the studied temperate, mid-latitude forests, are not the main regulating factor of the reproductive activity in males of *P. d. felipensis*. Young *et al*.[5] have shown under laboratory conditions that a combination of shorter daylight length, lower temperature and food restriction, reduced testis volume by apoptosis in *P. maniculatus*. Our results with free-living *P. difficilis* do not support the above since even though the temperature is lowered and daylight is shorter in fall-winter, both plant and insect food items are still available at these mid-latitude, temperate forests[44,45]. In such conditions, even though testicular size decreases, both testosterone production and spermatogenesis do not cease[25]; in fact, here we observed no apoptosis processes in the testicles and epididymis analyzed. Therefore, one alternative option is that both production of testosterone and of spermatozoa will decrease rather due to the diminishment of plant secondary compounds (phytoestrogens, FTE) or fungi-derived compounds (mycoestrogens, MCE), during food intake[46-48], when these food resources become scarcer o disapear.

Both FTE and MCE can mimic mammal estrogens and, thus depending on their amounts, they could deplete or enhance reproductive physiology in wild mammals[49,50]. At high quantities, they can disrupt normal on-off controls of steroid hormones in herbivorous mammals, thus inhibiting their non-genomic and genomic effects[51-53]. On the other hand, at lower amounts, FTE and MCE could trigger reproductive physiological processes for mating and reproduction[54]. Therefore, FTE and MCE could regulate reproductive activity in wild herbivores[55,56]. Accordingly, there is a relation between the reproductive activity of free-living, rodent species and the time of year when food is abundant and thus of FTE and MCE contents. For instance, Jameson Jr. and Peeters[57] reported an increase of reproductive activity in heteromyid mice from a desert: during the summer rains, coinciding with the rise of plants, mice consumed more phytochemicals (e.g., phytoestrogen), while with the reduction of such food resources, during the dry season, mice underwent a delayed and decreased reproductive activity. Kaneko[58] found that litter size decreased (36.7%) in a population of Japanese voles (*Microtus montebelli*) inhabiting a plantation of cypresses, thereof containing a high concentration of phytoestrogens, as compared to another population inhabiting a fallow rice field. For its part, Berger *et al*.[59] found delayed oogenesis and decreased uterine size in females of *Microtus montanus*, due to compounds

contained in plants eaten during late summer, especially during the dry years; therefore the reproductive activity of this species may be also affected by phytochemicals present in plants they consume[60]. In our study area, it is suggestive that *P. d. felipensis* and its syntopic congeneric, *P. melanotis*, eat a high amount of fungi together with a larger diversity of plants during the rainy season[44,45], which might be stores of MCE and FTE that might be acting as triggers for breeding. Nevertheless, further studies are needed to elucidate whether FTE and MCE in food have any roll on reproduction in our model *Peromyscus*, as well as in another species of wild micromammals[61,62].

Also interestingly is the fact that bodyweight, deprived of testicular volume, was significantly reduced in adult males of *P. d. felipensis* during spring, the season with higher reproductive activity (Fig. 1b). Conversely, body weight increased during seasons with shorter days (fall-winter) and lower temperatures (Fig. 1b,c). Low weight may be due to high energy expenditures, together with physiological distress, caused by the increase of allostatic overload during reproductive events[63]; on the other hand, its subsequent increase, during fall-winter, might be due to an increase of metabolic rate during the cold seasons, to withstand the cold. We have found, indeed, that triglyceride content increases during colder seasons (autumn-winter) in free-living *Peromyscus melanotis* and *P. difficilis*[64]. Furthermore, the diet composition of adult males of *P. d. felipensis*, the intake of a lower amount of plant species is completed by arthropod consumption during the "harsher" environmental conditions[44,45]. The above could allow this species to gain weight (e.g., muscle mass and fat tissue) during the autumn-winter to prepare it for the allostatic overload derived from the search and copulation with mates[65], as well as for the defense of the mating territory, as has been reported for other wild mice[8,66-68].

Finally, we have shown here that male reproductive activity of free-living *P. difficilis felipensis* is affected by coincident internal and external conditions that then determine individual reproductive success. Constant sperm in testis tubules and the epididymis, together with adult male testosterone production and intra-gonadal testosterone content, associated with daylight and temperature oscillations, are only part of the population maintenance and perpetuation. However, other involved environmental factors also wait to be evaluated, to establish their role in the reproductive dynamics of free-living, herbivore mice such as *P. d. felipensis*. One is the obvious need for documentation on the basic female reproductive physiology. Another aspect of particularly important interest is the study of potential sex hormone-like acting compounds (e.g., FTE and MCE) within their diet, for these food compounds could be affecting the whole population's physiological reproductive cycle and might ultimately regulate perpetuation of *P. d. felipensis*, as well as that of other rodents sharing this mid-latitude, temperate forest habitat.

CONCLUSION

In adult males of *Peromyscus difficilis felipensis* from mid-latitude, temperate forests, spermatogenesis occurred all year round, but their testis function (testis volume, testosterone production and intra-gonadal testosterone content) and reproductive peak were enhanced by longer day length and higher temperatures. Fluctuations of body weight (decrease in spring-summer; increase in fall-winter) were also related to its reproductive pattern. Rather than physical variables, in habitats ruled by the rainy seasons, the more food availability and diversity (e.g., more plants and fungi food items) associated with contents and amount of plant and fungi chemicals, might promote such patterns.

SIGNIFICANCE STATEMENT

To avoid underestimations of the reproductive activity in the population of wild, adult male mice with a retractile testis, it is necessary to verify that their size and position agree with other physiological parameters such as the production and contents of sex hormones. In addition to abiotic factors such as daylight length and temperature, the testicular function of mid-latitude, temperate mice may be being triggered and sustained by bioactive compounds consumed in plant and fungi food items.

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