at 20.30 h (E.D.S.T.) shows $r = 0.768$ ($p < 0.05$) for the spring flight, $r = 0.664$ ($p < 0.01$) for the summer flight and $r = 0.908$ ($p < 0.001$) for the pooled data. Similarly, wild *H. immaculata* males were lured to synthetic 2-methylheptadecane, the female sex pheromone[16], for the 2 h prior to sunset on a cool day (a high of 24 °C) and for 2 h after sunset on a warm day (a high of 30 °C)[4].

In the oscillating daily temperatures found in nature these rhythmic modifications of the mating clock could be a function of the daily thermoperiod or they could be initiated by ambient temperature fluctuations occurring during a specific daily interval or selective circadian gate. The interactions of temperature and light cycles in affecting the initiation and duration of female calling behavior and male responsiveness are complex. Notwithstanding, in *A. velutinana* a decrease in temperature occurring within a specific daily gate can induce both female calling and male responsiveness within a few minutes (Table). Even if this decrease in temperature is as little as 1 °C, a significant number initiate mating behavior. In the laboratory, 14.3% of the females ($N = 210$) experiencing a decrease from 24° to 23 °C 1 h prior to lights-off in a 16:8 LD commenced calling behavior within 10 min [where the 95% confidence interval (CI) extends from 9.5 to 19.3%]. In the control group 0.5% of the females were calling (a CI to 2.6%). In male pheromone bioassays ($N = 200$) 54.3% experiencing an identical decrease in temperature were responsive to female pheromone extract (CI 47.4 to 61.3%), whereas only 24.5% control males were responsive (CI 18.9 to 31.0%).

These findings indicate that the control centers for female calling behavior and male pheromone responsiveness have inputs from the circadian clock as well as external stimuli, apparently unlike the periodicities of female calling behavior and male flight in *A. pernyi* which are tightly coupled to the output of the clock[10]. This is the first report of current conditions modifying the timing of a circadian rhythm without appearing to affect its entrainment.

The adaptive significance of this intricate, daily temporal coordination of mating rhythm with temperature is obvious: cool temperatures are undesirable because they increase the demand for metabolic energy necessary to sustain mating flight[17]. This consideration is most crucial in small insects (such as *A. velutinana*) that possess a high surface area to volume ratio. Operating against this factor are possible evolutionary disadvantages such as increased difficulty in maintenance of temporal partitioning of closely-related species that utilize a common chemical communication system[1–3] and exposure to additional predator pressure during diurnal activity.

*Résumé.* Les lépidoptères modifient leur rythme endogène d'activité sexuelle selon le régime de température du jour. Les fluctuations de température dans certaines parties du cycle photopériodique servent de signal pour déterminer l'heure de l'accouplement.

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## The Rate of Testicular Development in Japanese Quail (*Coturnix coturnix-japonica*) Following Stimulation of the Extra Retinal Photoreceptor

Seasonal reproduction in many birds is regulated by the annual change in daylength, with the longer photoperiods of spring and summer causing increased gonadotrophin secretion. Knowledge about the neuroendocrine basis of this photoperiodic response is quite extensive[1,2] but our understanding of the sensory structure(s) involved in light detection, and of the mechanisms used for measuring its daily duration[3] is much more limited. The issue of light perception has been especially intriguing ever since Benoit suggested in the thirties that an extra-retinal photoreceptor existed in the duck. His many experiments led him to conclude[4] that the eye played little or no role in the photoinduction of testicular growth and that another photoreceptor must exist elsewhere in the brain, probably within the hypothalamus. More recent studies in the house sparrow by MENAKER et al.[5–8] confirm these findings and they have concluded that the eyes do not participate at all in the reception of light for the photoperiodic response. This is possibly true in other species also[9–11].

In the Japanese quail (*Coturnix coturnix japonica*) there is clearcut evidence that enucleation does not block photoinduced gonadal growth[12,13] or lead to regression in sexually mature birds maintained on long daylengths[14–16].

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Again, therefore, the eyes do not seem to be involved. Support for this comes from the use of radioluminous paint (RLP) which can be used to stimulate the extra-retinal receptor directly. The application of this material to the skull of sexually mature quail prevented the testicular atrophy normally seen when birds are transferred to short days[17], while plates of the paint placed close to the hypothalamus stimulated gonadal growth in birds held in non-stimulatory conditions[18].

One aspect, however, is not clear from the quail experiments. Can the extra-retinal receptor sustain a rate of gonadal growth similar to that seen in untreated birds exposed to long days? This has been answered only in the sparrow where testicular growth curves are identical in both blinded and intact photostimulated birds[8]. In the present experiments a somewhat different approach has been used. The rate of testicular development has been compared between birds exposed to continuous light (LL) and birds maintained on short day-lengths but where the extra-retinal receptor has been stimulated by radioluminous paint. If quail are photostimulated the relationship between testicular weight and the number of days of treatment is loglinear (i.e. $\log_{10}W_t = \log_{10}W_o + kt$, where $W_o$ is the testicular weight at the outset and $W_t$ the weight after $t$ days of stimulation[19]). The slope of this line ($k$) is a quantitative measure of the rate of testicular growth.

*Materials and methods.* Japanese quail were reared from hatch under short daylengths (8 h light/16 h dark; 8L/16D) and used at exactly 5 weeks of age when they were approaching somatic maturity. Orange and green radioluminous paints (Atomloihi No. 3000) were purchased from Dai Nippon Sinloihi Co. Ltd., Chukoku, Tokyo. They had emission maxima at 600 n$M$ and 520 n$M$ respectively. A weighed amount was mixed with a drop of binding agent and coated onto white polythene discs (10 mm diameter). Discs containing only the binding agent were used as controls. The discs were sutured in place on the surface of the skull. After surgery the quail with implants of RLP were returned to 8L/16D. Control groups were placed on LL, or retained on 8L/16D. Quail were killed after varying lengths of time and at autopsy the body and combined testicular weights were measured. In some cases plasma LH was estimated by radioimmunoassay[20].

Table I. Testicular weight and plasma LH after 12 days treatment with various amounts of radioluminous paint

| Treatment | Combined testicular weight (mg) | Plasma LH (ng/ml) |
|---|---|---|
| 3 mg orange RLP[a] | 20.5 ± 2.4 (6)[b] | — |
| 6.25 mg orange RLP | 31.6 ± 7.3 (6) | — |
| 12.5 mg orange RLP | 42.4 ± 12.2 (6) | 1.2 ± 0.4 (5)[b] |
| 25 mg orange RLP | 516 ± 91 (10)[c,d] | 2.5 ± 0.5 (5)[d] |
| 12.5 mg green RLP | 29.4 ± 6.4 (5) | — |
| 25 mg green RLP | 38.0 ± 8.1 (12) | 0.9 ± 0.3 (6) |
| 50 mg green RLP | 24.8 ± 5.0 (4) | 1.0 ± 0.2 (4) |
| Binder only | 36.0 ± 8.0 (16) | 0.7 ± 0.1 (5) |
| LL controls | 635.0 ± 36.4 (11)[c] | 3.5 ± 0.8 (5)[d] |
| 0 day controls | 16.3 ± 2.0 (10) | 0.6 ± 0.1 (7) |

[a] RLP is an abbreviation for radioluminous paint. [b] Mean ± SEM. No. of animals in parentheses. [c] $P < 0.001$ compared with binder only group. [d] $P < 0.05$ compared with binder only group.

*Results.* A first experiment attempted to establish whether there was a relationship between the quantity of RLP used in an implant and the subsequent amount of testicular growth. Groups of quail were implanted with discs containing orange or green paint, or binder alone, and were killed after 12 days treatment. The results are summarized in Table I. In those exposed to the orange RLP, only the group with 25 mg implants showed any testicular growth ($p < 0.001$ compared with 0 day controls, or binder-only group). The plasma LH concentration was also elevated in this group. Although there was much variation in testicular size the mean value was not different from that seen in the LL controls ($p > 0.2$). The green paint was without effect although 1 bird with a 25 mg implant did show some growth (testicular weight 110 mg).

The second experiment compared further the growth rate in quail bearing 25 mg orange RLP implants with that seen in LL. Control groups on 8L/16D were also included. Samples were taken after 0, 7 and 18 days of treatment. The results, together with the 12-day data from the first experiment, are shown in Table II. Some small amount of growth occurred in the 8L/16D controls but this is normal for domesticated strains of quail. The birds exposed to LL or to light from the implant grew their testes much more dramatically and after 18 days were nearing sexual maturity. The mean testicular weight in the orange RLP group was significantly less than in the LL controls after 7 days treatment ($p < 0.05$) but this was not so at the other times. The calculated rates of testicular growth ($k$) for the 3 groups (with their 95% confidence limits) were: Orange RLP $k = 0.116$ (0.096–0.135); LL controls $k = 0.122$ (0.112–0.133); short day controls $k = 0.042$ (0.024–0.065). The rates were similar ($p > 0.50$) in the orange RLP and LL groups but both were highly significantly different ($p \leq 0.001$) from the rate under short days.

Plasma LH levels were estimated in the various treatment groups with the following results: 0 day group 0.6 ± 0.1 (SEM) ($n = 6$) ng/ml; 7 days, orange RLP 3.2 ± 0.8 (5) ng/ml, LL group 3.4 ± 0.3 (6) ng/ml, 8L/16D controls 0.6 ± 0.1 (4) ng/ml; 12 days, see Table I; 18 days, orange RLP 4.0 ± 0.4 (4) ng/ml, LL group 3.8 ± 0.3 (4), 8L/16D controls 1.1 ± 0.3 (5) ng/ml. As expected the LH levels were increased in the groups under LL or bearing the orange RLP implants.

*Discussion.* The experiments confirm those of Homma and Sakakibara[18] that direct illumination of the brain with radioluminous paint can induce testicular growth under non-stimulatory photoperiods. They complement the studies where the paint has been used to maintain testicular size following the transfer of mature birds to short daylengths[14,16]. More importantly, however, the results indicate that the extra-retinal receptor can sustain a rate of growth indistinguishable from that seen under continuous light. This argues that the quail – like the house sparrow[8] – probably does not use its eye as a photoreceptor for the photoperiodic gonadal response. The receptor clearly has a threshold since lower doses of orange RLP were unable to stimulate it.

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Table II. Comparison between testicular growth induced by orange radioluminous paint and continuous light

| Treatment | Combined testicular weight (mg) after the following number of days of photostimulation | | | |
|---|---|---|---|---|
| | 0 | 7 | 12 | 18 |
| Short days | 14.9 ± 1.3 (16) | 16.9 ± 2.5 (5) [b] | 24.9 ± 3.1 (23) [b] | 77.4 ± 23.6 (6) |
| Continuous light | 14.9 ± 1.3 (16) | 208 ± 33 (8) | 635 ± 36 (11) | 2153 ± 319 (8) |
| 25 mg Orange RLP [a] | 14.9 ± 1.3 (16) | 91 ± 20 (5) | 516 ± 91 (10) | 2290 (2) |

[a] RLP is an abbreviation for radioluminous paint. [b] Mean ± SEM. Number of animals in parentheses.

Green radioluminous paint was without effect on gonadotrophin secretion. This is perhaps not too surprising as red/orange light is much more effective than blue/green light in inducing gonadal growth[4,16,21]. Whether this reflects the spectral responsivity of the photoreceptor or merely the greater tissue penetrance of the longer wavelength red light is unknown. The present results say nothing as to the site of the extra-retinal receptor. Other evidence, however, argues against it being the pineal gland[12,15,16,18,22] although this organ might play a minor role in quail[23]. This does not of course exclude a function for the pineal in other species[24]. A final caution must be entered as HOMMA et al.[13] have recently reported some experiments in quail where they claim that the eyes do have a role in the photoperiodic control mechanism. They might be necessary for birds to distinguish short days under some circumstances for enucleation does not always lead to gonadal regression when birds are transferred from 18L/6D to 8L/16D.

*Résumé.* Pour stimuler le photorécepteur extrarétinal de cailles japonaises (*Coturnix coturnix japonica*), des disques enduit de couleurs radiolumineuses ont été introduite sous la peau de leur crâne et soumis à un éclairement de courte durée en lumière naturelle. A une dose de 25 mg, une teinte maintenue dans la région de l'orange de spectre provoque un notable accroissement testiculaire. Le taux de cette augmentation ne différa pas de celui qui s'observe chez la caille soumise à un éclairement continu. Des enduits émettant la couleur verte furent sans effet. Ces données laissent supposer que la caille n'utilise pas son œil comme photorécepteur stimulant le développement périodique de ses gonades.

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## Increased Fusion Frequency of *Aspergillus nidulans* Protoplasts

We earlier reported that protoplast fusion and heterokaryon formation could be achieved with auxotrophic mutants of *Geotrichum candidum*[1]. Nutritional complementation occurred in low frequencies, depending on the conditions. It was further mentioned that protoplast fusion could also be attained with nutritionally-deficient *Aspergillus nidulans* mutants. We describe here the aggregation and an increased fusion frequency of *Aspergillus nidulans* protoplasts.

*Materials and methods.* Nutritionally-deficient stable UV-mutants of the strain *Aspergillus nidulans* paba1, y, ts6[2-3] were produced, and the mutants requiring lysine (lys) and methionine (met) were used in these experiments. The mutants were cultivated and maintained on culture medium containing 0.5% yeast-extract, 1% glucose and 2% agar, at pH 6–6.2.

If not otherwise stated, the medium for protoplast formation consisted of 0.6 *M* KCl in McIlvain citrate-phosphate buffer (pH 6.0), containing 1% freeze-dried digestive juice of the snail *Helix pomatia*. This enzyme solution was filtered through a membrane sheet (pore size 0.22 µm) to exclude particles deleterious to aggregation and to fusion of protoplasts. For protoplast fusion the same solution was used, without snail enzyme. For regeneration of protoplasts use was made of a minimal medium ($(NH_4)_2SO_4$, 5 g; $KH_2PO_4$, 1g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; glucose, 10 g in 1000 ml distilled water) supplemented with vitamins[4], KCl (0.6 *M*) and agar (2%). The same medium with added L-lysine and L-methionine (each 50 µg/ml) was employed to determine the number of colony-forming units and the frequency of protoplast fusion.

In order to cultivate the auxotrophic mutants under optimum conditions for growth and protoplast production, a modification of the previously-described cellophane-

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