

Tests of absolute photorefractoriness in four species of cardueline finch that differ in reproductive schedule

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Summary

Species with different reproductive schedules may differ in how they respond to the annual change in photoperiod (the adaptive specialization hypothesis). Seasonally breeding species are predicted to use photorefractoriness to terminate reproduction prior to inclement conditions in autumn and winter, whereas opportunistically breeding species may not exhibit photorefractoriness. We tested for absolute photorefractoriness in four species of cardueline finch that differ in their reproductive schedules: opportunistically breeding red crossbills, flexibly breeding pine siskins, and seasonally breeding Cassin's finches and gray-crowned rosy-finches. Field observations indicated that all four species regress their gonads and begin

prebasic feather molt in late summer or autumn. However, exposure to a long day photoperiod in autumn (24 h:0 h L:D) resulted in elevation of gonadotropins and testicular recrudescence in all species except Cassin's finches. Thus, by this criterion, some of the seasonally breeding species tested here did not exhibit absolute photorefractoriness. These results indicate that phylogenetic history needs to be taken into account when considering the adaptive nature of photoperiod response systems.

Key words: photoperiod, reproduction, *Loxia curvirostra*, *Carduelis pinus*, *Carpodacus cassinii*, *Leucosticte tephrocotis*.

Introduction

Animals are naturally selected to reproduce at times of year when offspring are most likely to survive (Baker, 1938; Lack, 1968; Perrins, 1970). The primary environmental cue used by seasonally breeding songbirds to time gonadal development for spring breeding is the annual change in photophase, or day-length (Dawson et al., 2001). In these species, reproductive development is initiated by the vernal increase in photophase and terminated by the onset of photorefractoriness (Nicholls et al., 1988). During photorefractoriness the brain no longer responds in a stimulatory fashion to photoperiods that had previously been stimulatory and the hypothalamo–pituitary–gonad (HPG) axis is downregulated (Ball and Hahn, 1997; Nicholls et al., 1988). Exposure to short days may be required to break photorefractoriness such that birds are once again photosensitive to the stimulatory effects of long days in spring.

In seasonally breeding songbirds photorefractoriness is typically absolute. The absolute nature of this photorefractoriness has been defined in two ways (Hamner, 1968; Farner et al., 1983; Nicholls et al., 1988). First, in species that become absolutely photorefractory the gonads will regress spontaneously when held on constant long days. Second, once gonads have regressed birds are completely unresponsive to very

long days, even to 24 h of light. These two criteria have been used interchangeably to characterize the state of absolute photorefractoriness, and indeed in many species both phenomena occur. There is no *a priori* reason to assume, however, that these two phenomena may not be dissociated in some species.

Species vary in the timing of their reproduction, and it has been proposed that interspecific variation in the timing of breeding may result from interspecific variation in the systems that respond to photoperiod (Ball and Hahn, 1997; Coppack and Pulido, 2004; Dawson et al., 2001; Hahn et al., 1997; Hau, 2001; Lofts and Murton, 1968). That is, photoperiod response systems may be adaptively specialized to appropriately start and stop reproduction at different times for different species (the adaptive specialization hypothesis). Alternatively, different breeding schedules could result from species with identical response systems living at different latitudes (the conditional plasticity hypothesis). Finally, species may have photoperiod response system characteristics inherited through phylogenetic descent that are selectively neutral in regards to reproductive timing. Rarely, however, are these latter two hypotheses considered in reviews of interspecific variation in breeding schedules and photoperiodism (see Hahn and MacDougall-Shackleton, 2006).

In this study we tested the photoperiod response systems of four species of cardueline finch [tribe Carduelini, Sibley and Monroe, Jr (Sibley and Monroe, Jr, 1990)] in order to test if opportunistic and flexibly breeding birds have reduced or eliminated photorefractoriness in comparison to strictly seasonal breeders. The species used were red crossbills *Loxia curvirostra*, pine siskins *Carduelis pinus*, Cassin's finches *Carpodacus cassinii* and gray-crowned rosy-finches *Leucosticte tephrocotis*. These species range from opportunistic to strictly seasonal. We used field observations to determine if birds regress gonads while day lengths are still long (criterion 1). We experimentally tested criterion 2 of absolute photorefractoriness: failure to respond to very long photoperiods (24 h:0 h L:D) when putatively photorefractory (regressed gonads and undergoing molt). Below we review the breeding schedules of the four species used, and predicted outcomes of the adaptive specialization hypothesis.

Red crossbills

Red crossbills are nomadic and are opportunistic breeders capable of reproducing at any time of year as long as the conifer seeds that they feed their young are abundant (Benkman, 1990; Newton, 1973). However, they do typically regress their gonads and undergo feather molt in autumn (Hahn, 1998). Thus, it appears that opportunistic breeding in this species is overlain on a fundamentally seasonally pattern of reproduction (Hahn, 1998). Research with captive crossbills suggests they do not become absolutely photorefractory by criterion 1 (Hahn, 1995). The adaptive specialization hypothesis would predict that crossbills retain the ability to respond to environmental cues (including long days) even when gonads have regressed and feathers are molting, as an adaptation to facilitate opportunistic fall breeding.

Pine siskins

Pine siskins are a nomadic irruptive species with a relatively long breeding season across a wide latitudinal and altitudinal range (Dawson, 1997). Despite their long and flexible breeding cycle, pine siskins do become photorefractory by criterion 1 (Hahn et al., 2004). The adaptive specialization hypothesis would predict that siskins may retain responsiveness to environmental cues (including long days) in order to facilitate a long, flexible breeding season.

Cassin's finch

Cassin's finches are high altitude, strictly seasonal breeders (Hahn, 1996). Cassin's finches held on constant long days (15 h:9 h L:D) eventually regress their cloacal protuberance [an androgen dependent trait (Schwabl and Farner, 1989)] and undergo prebasic feather molt (T.P.H., unpublished data). Congeneric house finches *Carpodacus mexicanus* become absolutely photorefractory by both criteria (Hamner, 1968). The adaptive specialization hypothesis would predict that Cassin's finches become absolutely photorefractory to terminate breeding in late summer.

Gray-crowned rosy-finches

Gray-crowned rosy-finches are high altitude and/or high latitude specialists. High altitude populations breeding in the Sierra Nevada are strictly seasonal breeders with a relatively short breeding season (MacDougall-Shackleton et al., 2000). Although no data are available on this species' reproductive response to photoperiod the adaptive specialization hypothesis would predict that, because of their extreme breeding environment and short breeding season, rosy-finches should become absolutely photorefractory to terminate breeding in summer.

Materials and methods

We conducted descriptive studies of free-living male pine siskins *Carduelis pinus* Wilson 1810, gray-crowned rosy-finches *Leucosticte tephrocotis* Swainson 1832, Cassin's finches *Carpodacus cassinii* Baird 1854 and red crossbills *Loxia curvirostra* L. to document seasonal changes in reproductive condition in the wild. In addition, we performed captive photoperiod experiments (24L challenges) on these species to determine if they become absolutely photorefractory by criterion 2 (insensitivity to long days while putatively photorefractory). Finally, we interpret species differences in relation to phylogenetic relatedness.

Field methods

All animals in this study were cared for in accordance with Princeton University's Animal Care Committees and under permission of relevant state and federal permits. Field observations of siskins, rosy-finches and Cassin's finches were conducted in the summers of 1997 through 1999 in the vicinity of Tioga Pass in the Sierra Nevada of California (37°50'N; 119°12'W, 3000 m elevation). Field observations of red crossbills were conducted from 1987 through 1991 in Washington State at the following locations: Shaw Island, San Juan County, WA, 48°33'N, 122°57'W, 20 m elevation; Devils Table, Yakima County, WA, 46°48'N, 121°2'W, 1040 m elevation; Neilton, Grays Harbor County, WA, 47°25'N, 123°54'W, 100 m elevation.

Birds were captured in mist nets or walk-in traps baited with seed, salt or a caged decoy bird. Following capture, we measured the length of the cloacal protuberance and length of the left testis *via* laparotomy. We measured the size of the cloacal protuberance from the base of the protuberance to the cloacal opening to the nearest mm using dial callipers. Testis size was measured *via* laparotomy. Birds were anaesthetized with methoxyflurane (Metofane) vapors, then a small incision was made in the left flank and the length of the left testis measured to the nearest 0.1 mm by locking the tips of forceps on either side of the testis, then measuring the distance between the forceps tips with dial callipers. We also assessed the stage of prebasic molt by counting the number of growing primary flight feathers and the presence of new body pinfeathers.

In addition to field observations, we conducted experimental studies with birds from each of the four species. Birds used in

these studies were captured at Tioga Pass in the summers of 1998 and 1999 and were held captive under natural photoperiod at the field site for up to 3 months before being transported to Princeton University for the 24 h light (24L) challenge experiments (see below).

24 h light challenges

The photoperiod experiments tested whether birds of each species would respond to a 24L challenge during the time they were molting feathers and were potentially photorefractory. In Experiment 1 we tested whether birds were absolutely photorefractory by performing a 24L challenge when birds were nearing completion of primary feather molt. In Experiment 2 we repeated the test of absolute photorefractoriness with another group of birds that were early in the process of primary feather molt.

Experiment 1

This experiment used eight red crossbills ['Type 4' (Groth, 1993); type identified by vocalizations and morphological measurements], ten pine siskins, eight Cassin's finches and six gray-crowned rosy-finches. Birds were captured in May and June 1998 and were held in group cages at the field site until August. They were then transported to Princeton University and housed in outdoor aviaries until the experiment began. All birds were thus exposed to naturally changing photoperiod until the experimental manipulation. Throughout the study birds were provided *ad libitum* access to a diet of Mazuri small bird maintenance diet (PMI Nutrition, Henderson, CO, USA) supplemented with seeds, fresh greens and carrots.

On 17 October 1998 the birds were moved into experimental chambers. These acoustic isolation chambers (customized Industrial Acoustics Mini-booth 250, Bronx, NY, USA) had three shelves lit by vertically oriented fluorescent light fixtures. Birds were housed one or two birds per cage, with two cages per shelf. Only a single species was housed within a chamber, with two chambers per species. Thus all birds could see and hear other birds of their own species.

Each chamber had an electronic timer to control lights on and off. Initially the light schedule was altered daily to mimic the naturally declining day-length. On 23 October 1998 we randomly selected half the birds of each species (one of the chambers per species) and transferred them to a photoperiod of 24 h: 0 h L:D. The other half of the birds remained on the natural short day photoperiod of 10.8 h: 13.2 h L:D. Blood samples were collected from each bird 4 days prior to, and 3 and 10 days following, this switch in photoperiod. Blood was collected from the alar vein in heparinized microhematocrit tubes, centrifuged, and the supernatant plasma stored at -20°C until the hormone assay was performed. Ten days following the switch in photoperiod all birds were euthanized with an overdose of methoxyflurane vapors, the left testis was dissected out, its length measured to the nearest 0.1 mm using dial callipers, and the brains collected for other studies.

Hormone assay

Following the experiment all plasma samples were assayed for luteinizing hormone (LH) in a post-precipitation double-antibody radioimmunoassay (see Follet et al., 1972; Follet et al., 1975; Sharp et al., 1987) (see also Hahn et al., 2004). This assay has been used in a variety of songbird species. The assay used purified chicken LH as the standard and for iodination by the chloramine T method. Duplicate 20 μl plasma samples from the birds were run in a single assay to eliminate inter-assay variation.

Experiment 2

Birds that responded to the 24L challenge in Experiment 1 may not become absolutely photorefractory, or may have been absolutely photorefractory earlier during the molt process. Experiment 2 was carried out to test this second hypothesis. We captured eight male red crossbills (Type 4), eight male pine siskins and eight male gray-crowned rosy-finches in May and June of 1999. These birds were treated identically to those in Experiment 1 prior to the photoperiod manipulation. On 6 September 1999 the birds were moved into experimental chambers and held on a naturally declining photoperiod. On 15 September we transferred a randomly selected half of the birds of each species onto a photoperiod of 24 h:0 h L:D while the other half remained on a photoperiod of 12.5 h:11.5 h L:D. 3 days prior to, and 10 days following this switch in photoperiod we measured the length of each bird's left testis to the nearest 0.1 mm *via* laparotomy.

Statistical analyses

For field observations we compared testis size for each species using unpaired *t*-tests for three of the species (2 times of year), and an ANOVA for crossbills (4 times of year). To test for seasonal changes in gonad size we also ran quadratic regressions to test whether day of year was significantly associated with testis size. For the captive experiments we used multi-way ANOVAs to determine main effects of species and photoperiod. In the cases of significant interaction terms we ran planned comparisons separately for each species using unpaired *t*-tests or ANOVAs. Tukey *post-hoc* tests were used to determine significant within-factor differences.

Results

Field observations

Free-living male Cassin's finches, pine siskins and gray-crowned rosy-finches appeared to reach breeding condition in May and then come out of breeding condition during July, as evidenced by regression of the testes and cloacal protuberance and onset of prebasic molt (Fig. 1). Pine siskins are reported to breed flexibly in some eruption years (Dawson, 1997), and have been observed with large gonads and active nests during September in Washington State (T. P. Hahn, unpublished data). However, during this study males appeared to come out of breeding condition near the end of July in all three years. Gray-crowned rosy-finches appeared to have the shortest duration

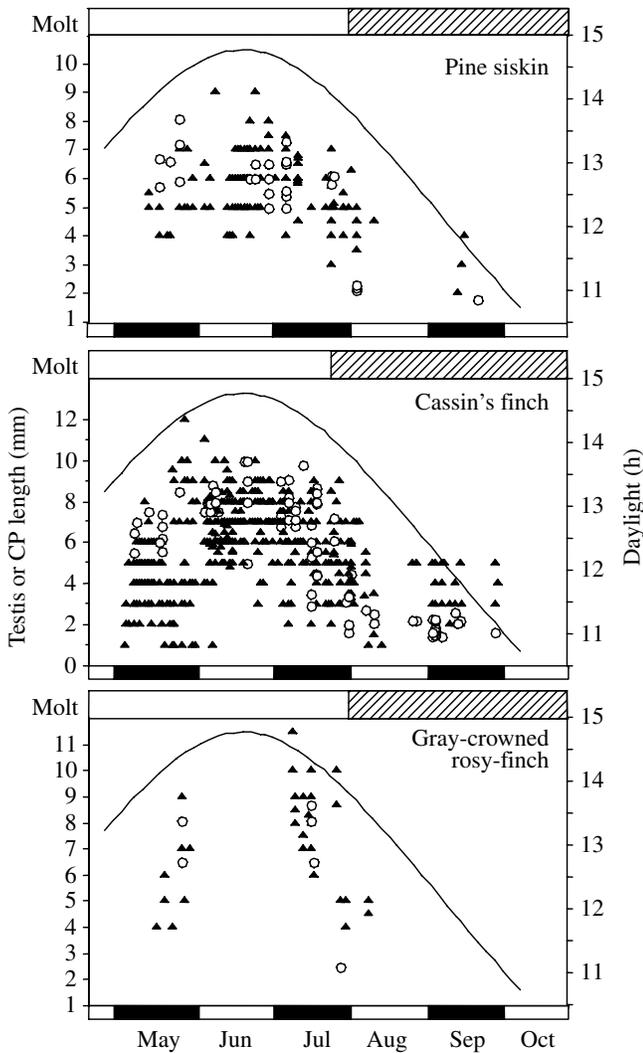


Fig. 1. Field data on seasonal changes in length of the left testis (circles) and cloacal protuberance (CP, triangles) in free-living pine siskins, Cassin's finches and gray-crowned rosy-finches breeding at Tioga Pass, California. The line indicates the number of hours of daylight. The hatched bars indicate when most birds were exhibiting feather molt. Testis length was measured in a subset of the birds for which we measured CP. Sample sizes are: pine siskin: 207 (CP), 31 (testis); Cassin's finch: 574 (CP), 82 (testis); gray-crowned rosy-finch: 31 (CP), 6 testis.

breeding season; however, we also collected the fewest field observations for this species.

Red crossbills are opportunistic breeders and routinely breed from January through September and they may occasionally breed October through December if conifer seeds, which they feed their young, are abundant (Adkisson, 1996; Benkman, 1990; Benkman, 1992). Our field observations are consistent with this (Fig. 2). Cloacal protuberance length varied little over the year. Testes could be large any time from January through September, were consistently large in summer when the birds sampled were actively breeding in areas with new developing cone crops, but were smaller for the autumn months October

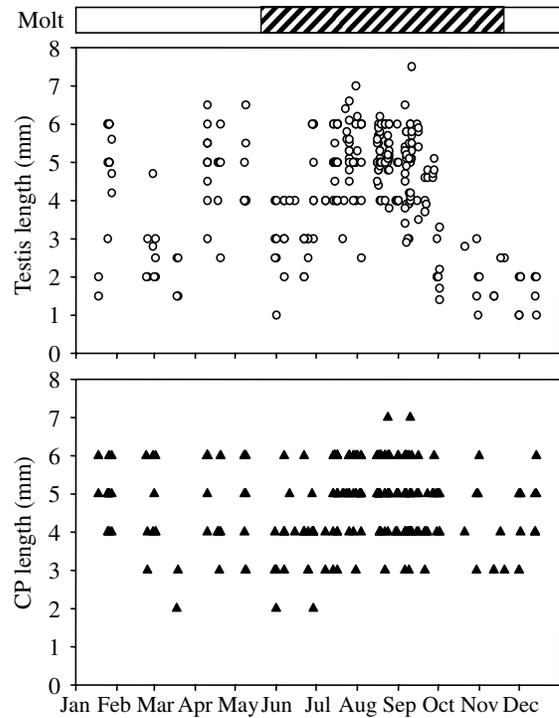


Fig. 2. Field data on seasonal changes in length of the left testis (circles) and cloacal protuberance (CP, triangles) in free-living red crossbills captured in the vicinity of Washington State. The hatched bars indicate when most birds were exhibiting feather molt. Testis length and CP were measured in the same birds. Sample size is 308 birds.

through December. Note that the relatively small maximum testis size of the red crossbills compared with the other three species does not reflect failure to breed, but rather the fact that male red crossbills never develop testes as large as many other songbirds (Hahn, 1998).

Photorefractoriness is often characterized by an asymmetry in the annual change in gonad size around the summer solstice, with some species even regressing their gonads prior to the summer solstice (Dawson et al., 2001). This pattern was not conspicuous in any of the species studied here. Close examination, however, suggests that at least Cassin's finches and pine siskins may exhibit an asymmetry in gonad size relative to the summer solstice. 1 month prior to the solstice (21 May), testes were growing, and were larger than they were 1 month after the summer solstice (21 July), at which time gonads were regressing (Fig. 1). To test for an asymmetry in testis size for Cassin's finches, pine siskins and rosy-finches we compared testis lengths for two time periods spaced equally before and after the summer solstice (8–26 May and 19 July–4 Aug; Fig. 3). The duration of daylight (sunrise to sunset) is equal for these two times, increasing from 14.0 to 14.5 h of light in May, and decreasing from 14.5 to 14.0 h light in July/August. For pine siskins and Cassin's finches, testes were significantly larger before the summer solstice than after (Fig. 3; pine siskin: $t_{10}=2.8$, $P=0.018$; Cassin's finch: $t_{19}=2.5$,

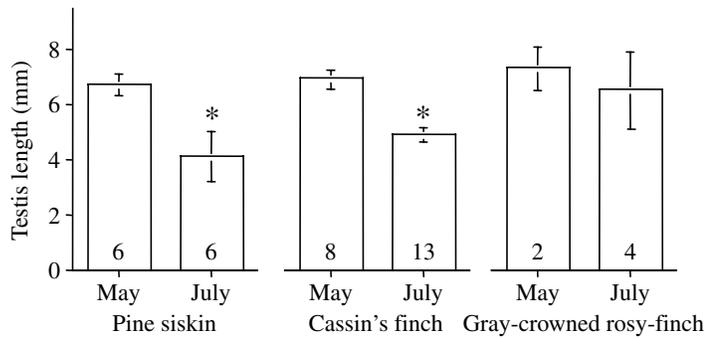


Fig. 3. Comparison of testis length of pine siskins, Cassin's finches and gray-crowned rosy finches across two times in the breeding season when day length varies between 14.0 and 14.5 h light (May: 8–26 May; July: 19 July–4 Aug) at Tioga Pass, California. Bars indicate mean \pm s.e.m. For *P* values, see text. Asterisks denote significant differences between times. Numbers within bars indicate sample size.

$P=0.022$). There was no significant difference in testis size between these two times for gray-crowned rosy-finches, though sample sizes were very small ($t_4=0.4$, $P=0.71$). Thus, at least Cassin's finches and pine siskins appear to become less responsive to long days as the breeding season progresses.

To examine potential seasonal changes in breeding condition in crossbills we used ANOVAs to compare testis length and cloacal protuberance length across the four seasons, demarcated by the vernal and autumnal equinoxes, and summer and winter solstices (Table 1). Both cloacal protuberance ($F_{3,304}=6.2$, $P<0.001$) and testis length ($F_{3,304}=57.9$, $P<0.001$) varied significantly across the year. Cloacal protuberance was significantly larger in summer (21 Jun–20 September) than in autumn (21 September–20 December) but no other differences were significant (Table 1). For testis size, all four seasons differed significantly from each other except winter (21 December–20 March) and spring (21 March–20 June; Table 1). Thus, reproductive condition in crossbills did vary across the annual cycle with peak cloacal protuberance and testis size following the summer solstice and smallest cloacal protuberance and testis size in autumn. Testis size increased in summer as day-length was declining – thus crossbills did not

Table 1. Seasonal variation in mean cloacal protuberance and testis size in red crossbills

Season	<i>N</i>	Cloacal protuberance length (mm)	Left testis length (mm)
Winter (21 Dec–20 Mar)	30	4.7 \pm 0.2 ^{a,b}	3.8 \pm 0.3 ^a
Spring (21 Mar–20 Jun)	47	4.7 \pm 0.2 ^{a,b}	4.3 \pm 0.2 ^a
Summer (21 Jun–20 Sep)	190	5.1 \pm 0.1 ^a	5.0 \pm 0.1 ^b
Autumn (21 Sep–20 Dec)	41	4.4 \pm 0.1 ^b	2.5 \pm 0.2 ^c

Individual data are plotted in Fig. 2. Values are means \pm s.e.m.; values indicated by the same superscript letter do not differ from each other (Tukey's *post-hoc* tests).

exhibit peak gonadal size prior to the solstice, as often occurs in species that become photorefractory.

Further evidence that gonad size changes seasonally in all four species was obtained by using a quadratic regression to model changes in testis size as a function of day of year. For all four species, time of year explained a significant proportion of variation in testis size (gray-crowned rosy-finch: $r^2=0.88$, $P=0.04$; pine siskin: $r^2=0.56$, $P<0.001$; Cassin's finch: $r^2=0.67$, $P<0.001$; red crossbills: $r^2=0.22$, $P<0.001$).

24L challenge, Experiment 1

In this experiment birds were challenged with 24 h light on 23 October. At this time all birds in the study were nearing completion of the prebasic molt. Most birds were growing their 8th and 9th primary feathers (range 5th to 9th primary) and had nearly completed body molt.

Exposure to 24 h:0 h L:D resulted in increased testis size in three of the four species (Fig. 4). A multi-way ANOVA revealed a significant main effect of species ($F_{3,24}=13.9$, $P<0.0001$), a significant main effect of photoperiod ($F_{1,24}=54.3$, $P<0.0001$), and a significant interaction between species and photoperiod ($F_{3,24}=13.9$, $P<0.0001$). *Post hoc* tests indicated that gray-crowned rosy-finches had larger testes than the other three species, which did not differ from each other. The significant interaction term indicated that different species responded to the change in photoperiod differently. Thus we compared birds exposed to 24L to those exposed to short day photoperiod separately for each species. Red crossbills ($t_6=4.6$, $P<0.01$), pine siskins ($t_8=6.7$, $P<0.0001$) and gray-crowned rosy-finches ($t_4=3.8$, $P<0.02$) exposed to 24L had significantly larger testes than those held on natural ambient photoperiod. This effect was most pronounced in gray-crowned rosy-finches where testis length of photostimulated birds was more than double that of short day birds (Fig. 4). In contrast there was no significant difference in testis length between Cassin's finches exposed to the two photoperiods ($t_6=-0.4$, $P=0.7$). Indeed, mean testis length was nearly identical in these two groups (Fig. 4). These results indicate that Cassin's finches were absolutely photorefractory at this time, but none of the other species were.

Levels of plasma luteinizing hormone (LH) are illustrated in Fig. 5. To assess changes in LH we ran a multi-way ANOVA to compare species, treatment group (short day *versus* 24L) and sampling day as a within-subjects factor. There were significant main effects of photoperiod group ($F_{1,24}=14.9$, $P<0.001$) and sampling day ($F_{2,48}=8.1$, $P<0.001$), but no significant main effect of species ($F_{3,24}=1.2$, $P=0.3$). *Post-hoc* analyses indicated that LH was significantly higher in birds exposed to 24L and on day 10 as compared to days –4 and 3, which did not differ from each other. In addition to these main effects, there were significant interactions among species, photoperiod and sampling day ($F_{6,48}=6.1$, $P<0.0001$). Thus, for each species we ran separate two-way ANOVAs with sampling date as a within-subjects factor and photoperiod as a between-subjects factor (Table 2). For pine siskins there was a significant effect of photoperiod, and sampling date, and a significant interaction

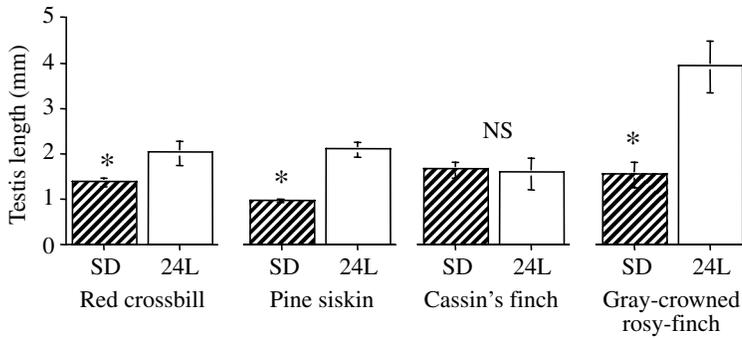


Fig. 4. Testis size of birds maintained on short days or transferred to 24 h light in Experiment 1. Bars indicate mean \pm s.e.m. For *P* values, see text. Asterisks denote significant differences; NS, no significant difference. Sample sizes are red crossbill: 4, 4; pine siskin: 5, 5; Cassin's finch: 4, 4; gray-crowned rosy-finch: 3, 3.

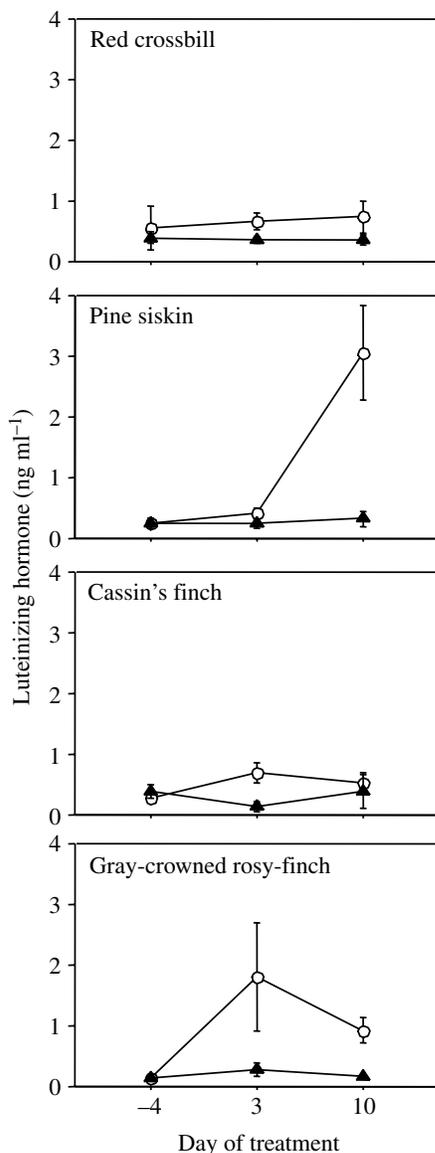


Fig. 5. Circulating levels of luteinizing hormone in birds exposed to short days (triangles) or 24 h light (open circles) 4 days prior to the change in photoperiod (d -4) and 3 and 10 days following it. Values are means \pm s.e.m. Sample sizes as in Fig. 4.

between these factors (Table 2). 10 days following the change in photoperiod siskins exposed to 24 h light had significantly higher levels of LH than birds held on short days. No other significant changes in LH were observed for the other species; however, a trend was observed for gray-crowned rosy-finches (Fig. 5). The small sample size for this species (3 birds per group) likely limited statistical power to detect the elevated LH of birds transferred to 24L.

24L challenge, Experiment 2

In Experiment 1, three of the four species tested were not absolutely photorefractory by criterion 2 during late prebasic molt. In Experiment 2 we tested for absolute photorefractoriness earlier, during prebasic molt. To do so we challenged birds with 24L approximately 1 month earlier than in Experiment 1, on 15 September. This date was selected as a point at which birds would likely have fully regressed gonads and would all be in the process of molt (see Fig. 1). However, only the latter turned out to be the case.

At the time of Experiment 2 all birds exhibited signs of prebasic molt. All birds except one pine siskin had dropped at least three primary feathers and most birds were molting body feathers as well. However, most of the rosy-finches still had some black pigmentation in their beaks, and two of them had fully black beaks characteristic of the breeding season (MacDougall-Shackleton et al., 2000). Laparotomies revealed that few of the birds had fully regressed testes, and in fact many had testes as large as seen during breeding in wild birds. Prior to the 24L challenge, testis length ranged from 1.2 to 3.1 mm for crossbills, from 0.8 to 6.9 mm for pine siskins, and from 1.4 to 7.8 mm for rosy-finches. To correct for this extreme heterogeneity, we calculated for each bird the size of the testis 10 days following the change in photoperiod relative to the its size prior to the change in photoperiod.

For all three species, birds maintained on natural ambient photoperiod continued to regress their testes whereas birds switched to long days grew their testes (Fig. 6). A two-way ANOVA indicated a significant effect of photoperiod ($F_{1,18}=7.7, P=0.01$): birds exposed to 24L for 10 days had larger relative testes than birds exposed to short days. There was no significant variation among species ($F_{2,18}=0.96, P=0.4$)

Table 2. Results of two-way ANOVAs comparing effects of photoperiod (24L versus natural photoperiod) and sampling date on plasma levels of luteinizing hormone

Species	Sample date	Photoperiod	Date × Photoperiod
Red crossbill	$F_{2,12}=0.1$	$F_{1,6}=1.3$	$F_{2,12}=0.4$
Pine siskin	$F_{2,16}=14.2^*$	$F_{1,8}=9.6^*$	$F_{2,16}=12.6^*$
Cassin's finch	$F_{2,12}=1.0$	$F_{1,6}=1.1$	$F_{2,12}=3.8$
Gray-crowned rosy-finch	$F_{2,8}=3.6$	$F_{1,4}=4.2$	$F_{2,8}=2.6$

* $P < 0.05$.
Data are illustrated in Fig. 5.

nor a significant interaction between species and photoperiod ($F_{2,18}=0.67$, $P=0.5$). Thus, exposure to 24L during the early stages of molt induced gonadal recrudescence in these three species.

Discussion

Interspecific comparisons often reveal a good match between variation in responses to photoperiod and variation in the timing of reproduction and migration (e.g. Lofts and Murton, 1968; Farner et al., 1983). This suggests that the photoperiod response systems of birds are adaptively specialized. That said, alternative hypotheses are possible (Hahn and MacDougall-Shackleton, 2006). For example, conditional plasticity may result in two species with identical photoperiod response systems exhibiting apparently specifically adapted breeding schedules at different latitudes (see S. J. Schoech and T. P. Hahn, manuscript submitted). As well, photoperiod response systems may exhibit features neutral to a current environment and be present through phylogenetic history.

In the present study we found limited support for the adaptive specialization hypothesis. As predicted, opportunistically breeding red crossbills and flexibly breeding pine siskins maintained responsiveness to environmental cues. With exposure to very long days these species were able to terminate gonadal regression and initiate gonadal recrudescence. In nature, this persistent responsiveness of the

HPG axis to environmental cues may permit flexible or opportunistic breeding in late summer or autumn if sufficient resources are available. Crossbills are well known to breed in mid-late summer when they locate abundant developing cones (Adkisson, 1996; Benkman, 1990; Benkman, 1992; Hahn, 1998; Hahn et al., 1997). Pine siskins also sometimes breed very late (August, September) using maturing conifer seeds, and as in crossbills this can apparently involve arrest of molt initiated following earlier spring breeding and re-acquisition of full reproductive competence (T.P.H., unpublished) (see also Dawson, 1997). We cautiously interpret the lack of absolute photorefractoriness by criterion 2 as an adaptive specialization in these species.

Similarly, the failure to respond to 24L exhibited by Cassin's finches could be interpreted as an adaptive specialization. Absolute photorefractoriness is generally considered an adaptation to terminate reproduction prior to the onset of inclement conditions in the autumn and winter. That said, because congeneric house finches and common rosefinches *Carpodacus erythrinus* also become absolutely photorefractory (Hamner, 1966; Hamner, 1968; Kumar and Tewary, 1982; Tewary and Dixit, 1983; Tewary et al., 1983) it is unclear if photorefractoriness is an adaptation for Cassin's finches or inherited as a neutral trait through phylogenetic descent within the *Carpodacus* genus.

The adaptive specialization hypothesis was not supported by data from gray-crowned rosy-finches. This species is an extreme environment specialist, breeding at very high altitudes or latitudes, and the subspecies tested here has a very short breeding season (MacDougall-Shackleton et al., 2000). If any of the taxa tested in this experiment needed a reliable mechanism to terminate reproduction before inclement autumn weather arrived, rosy-finches would make an excellent *a priori* candidate. Yet gray-crowned rosy-finches exhibited the most robust response to challenge with 24 h light of the four species we tested. Thus despite a short, strictly seasonal breeding season gray-crowned rosy-finches do not become absolutely photorefractory as defined by criterion 2. It remains to be determined if they would spontaneously regress gonads on constant long days (photorefractoriness criterion 1). There is currently no evidence that gray-crowned rosy-finches exhibit any form of photorefractoriness. Field data (Fig. 1) are consistent with a direct, seasonally symmetrical response to day-length with gonadal regression in late summer being driven by a decline in photoperiod beneath a seasonally invariant stimulation threshold, as in the woodpigeon (Lofts et al., 1967). Definitive evaluation of this hypothesis awaits future field and experimental studies.

The LH responses in this study were modest and appear to be somewhat delayed compared with those of fully

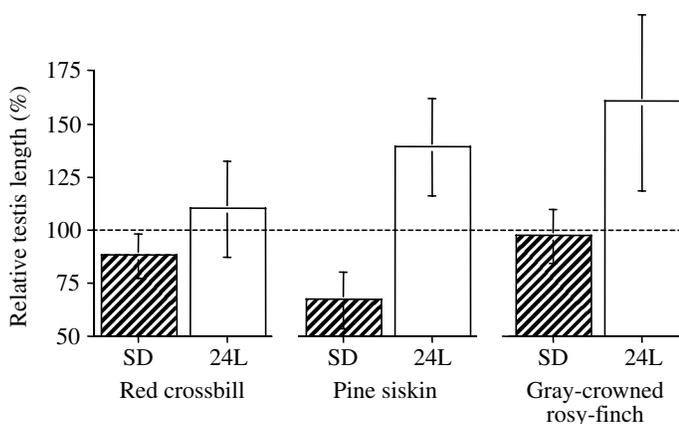


Fig. 6. Relative testis size of birds maintained on short days (SD) or transferred to 24 h light (24L) in Experiment 2. Bars indicate mean \pm s.e.m. Broken line indicates 100%, or no change in testis length. Sample sizes are 4 birds per group for all three species.

photosensitive birds in early winter, consistent with the interpretation that relative refractoriness (cf. Robinson and Follett, 1982; Nicholls et al., 1988) may have been present even if absolute refractoriness was not. Although the testes grew substantially with 10 days of 24L exposure, it is possible that gonadal recrudescence would be even more rapid at other times of year. Further experiments would be necessary to determine if these species are not photorefractory at all by criterion 2, or are only relatively photorefractory by this criterion.

Evaluation of adaptive specialization requires consideration of phylogenetic history. The phylogenetic relationships among the four species studied here are depicted in Fig. 7. This tree is consistent with several phylogenies of the carduelines, which indicate that pine siskins and crossbills are sister taxa, and that rosy-finches are more distantly related to the crossbills than are *Carpodacus* finches (Arnaiz-Villena et al., 2001; Badyaev, 1997; Marten and Johnson, 1986). The distribution of the two criteria for photorefractoriness for these species suggests that the presence or absence of photorefractoriness has been a labile trait during the divergence of the carduelines. Parsimony suggests that complete unresponsiveness to environmental cues when photorefractory (criterion 2) may be a derived specialization in the *Carpodacus* finches that was not present in the last common ancestor of all carduelines. Complete unresponsiveness when photorefractory is observed in other passerines such as European starling (Nicholls et al., 1988), so the potential lack of photorefractoriness in the last common cardueline ancestor may have been a derived specialization at that point in evolution. Though speculative, it is possible that this perpetual sensitivity to environmental cues may have preadapted the carduelines to the tremendous diversity in breeding schedules that the group displays.

The current study also indicates that the two criteria for photorefractoriness are dissociable traits. These two criteria have been assumed to reflect two characteristics of a unitary phenomenon (Nicholls et al., 1988). However, pine siskins, and perhaps other species, do spontaneously regress gonads when held on constant long days (Hahn et al., 2004), but are able to terminate molt and mount gonadotropic (Fig. 5) and gonadal (Figs 4 and 6) responses to stimulatory cues when putatively photorefractory. These data indicate that one criterion for photorefractoriness does not, therefore, necessarily imply the other.

In conclusion, our data indicate that phylogenetic history needs to be considered when determining whether photoperiod response systems are adapted to a particular species' breeding schedule. In addition, different criteria for photorefractoriness may vary among species relatively independently. Further work is clearly needed to resolve the evolution of photoresponsive mechanisms among cardueline finches. In addition to examining more basally related species such as evening grosbeaks (*Coccothraustes vespertinus*), we also need to determine responses to a range of photoperiods, as well as other cues, in order to quantify conditional plasticity. In combination these data may reveal how photoperiod response systems have adaptively responded to selection to permit a

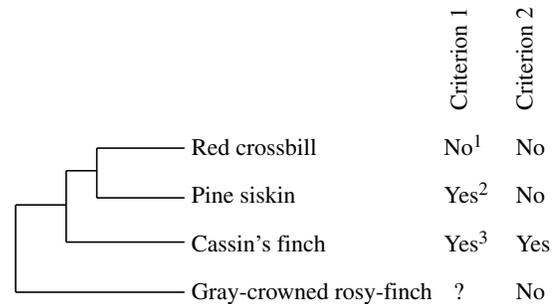


Fig. 7. Distribution of criteria 1 and 2 for absolute photorefractoriness among four species of cardueline finches. Criterion 1 indicates whether or not the species spontaneously regresses gonads on constant long days [data are taken from: ¹(Hahn, 1995); ²(Hahn et al., 2004); ³T.P.H., unpublished data (based on molt and cloacal protuberance)]. Criterion 2 indicates whether or not the species responds to very long days when putatively photorefractory (data from this study). Phylogeny based on Marten and Johnson, and others (Marten and Johnson, 1986; Badyaev, 1997; Arnaiz-Villena et al., 2001).

variety of breeding schedules, and how different taxa may respond to future environmental changes.

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