

Locomotor response of *Phreatichthys andruzzii* to chemical traces of unfamiliar conspecifics (A×B tests) and of *Barbus schuberti* (A×C tests): statistical analysis of results.

Test type	Intervals of analysis (min)	Experiments Positive	Neutral	Negative	Chi-square values	Significance
A×B	0–30	32	1	5	19.703	p < 0.001
	0–5	25	3	10	6.429	p < 0.02
	5–10	28	4	6	14.235	p < 0.001
	10–15	28	4	6	14.235	p < 0.001
	15–20	22	6	10	4.50	p < 0.05
	20–25	25	1	12	4.568	p < 0.05
	25–30	21	4	13	1.882	n.s.
A×C	0–30	20	0	18	0.105	n.s.
	0–5	23	1	14	2.189	n.s.
	5–10	18	4	16	0.118	n.s.
	10–15	19	5	14	0.758	n.s.
	15–20	17	4	17	0.00	n.s.
	20–25	12	5	21	2.455	n.s.
	25–30	17	4	17	0.00	n.s.

At present no hypothesis can be formulated about the nature of the attractive substance, except that this does not seem to consist of catabolism products presumably similar in both species. Moreover, the reaction does not appear to be dependent on food odor possibly present in the water of the home aquariums of the B and C groups. Furthermore, no patterns of food search were observed during the tests. Therefore the reaction appears to be dependent on some specific chemical signal. The biological significance of the presence of a species-specific response in a species which in the course of its evolution has undergone marked morphological regression and functional adaptation, and inhabits an environment free of any known sympatric species where presumably no selective pressure exists for its retention, has yet to be established. Nonetheless, it is further proof of both the complexity of the mechanisms regulating the oriented locomotory response in hypogean forms and the importance of chemical communication in their biology.

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Experimental evidence for endogenously programmed differential migration in the blackcap (*Sylvia atricapilla*)

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Summary. Female hand-raised blackcaps (*Sylvia atricapilla*) held in constant conditions (except for simulated seasonal changes in photoperiod) showed significantly more, and significantly longer, autumnal migratory activity as well as significantly later initiation of spring migratory activity than males from the same population (including siblings) held under identical conditions.

Key words. Migration; differential migration; endogenous program; blackcap; *Sylvia atricapilla*; warbler.

Migration is a relatively widespread attribute among both vertebrates and invertebrates that enables organisms to escape detrimental ecological conditions in one region while exploiting more benign conditions in another^{2, 3}. Migration, however, is a highly diverse behavioral phenomenon^{4, 5} and it has been well established that migratory behavior varies from highly facultative responses to immediate environmen-

tal change² to endogenously controlled behavior with high heritability values⁶. Of considerable interest from an evolutionary, ecological, and physiological standpoint are the relative roles of exogenous and endogenous factors in the regulation of migratory behavior^{7, 8}. Currently, the most persuasive experimental evidence for a high degree of endogenous regulation derives from an extensive, long-term investi-

gation of members of the genus *Sylvia* of Old World warblers, most notably the blackcap (*Sylvia atricapilla*)⁶. In these birds the timing, distance, direction and duration of migration have been demonstrated to be determined, to a large extent, endogenously^{6,7}.

'Differential migration' refers to the situation in which distinguishable classes of individuals (e.g., sex or age groups) differ with respect to timing, or distance, of migration and 'partial migrant' populations include some individuals that do, and some that do not, migrate from the breeding grounds⁹. There are two hypothetical explanations for these phenomena: 1) environmental regulation – there is a selective advantage to remaining at, or as close as possible to, the breeding grounds and subordinates are forced to migrate farther than dominants to have access to adequate resources during the nonbreeding season², and 2) endogenous control – individuals within a population differ with respect to their basic endogenous migratory programs⁶. Although these two hypotheses have been invoked to explain the same phenomena, it appears that both situations occur in nature and perhaps represent two ends of a behavioral continuum.

When sexual differential migration occurs in birds, it is almost without exception females that migrate farther (and arrive back on the breeding grounds later)², and information from field studies indicates that this is the situation in blackcaps^{10–12}. Since females are generally subordinate to males during the nonreproductive season in those species studied so far, this would seem to support hypothesis 1².

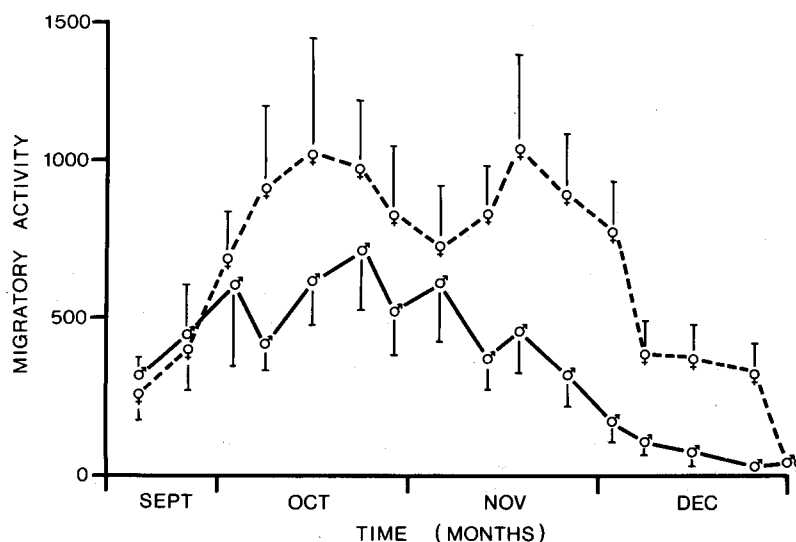
Indeed, many species that show pronounced patterns of differential migration are facultative migrants in which the stimulus to migrate appears to be primarily environmentally induced and is often not annual. However, differential migration is also found in populations in which annual, endogenously controlled migratory behavior is known to occur^{13,14}. In these populations the regulation of migratory behavior is much more controversial¹⁵. Here we report on experimental evidence that intersexual differential migratory behavior within an annual migrant population (all individuals migrate annually) has a sexually linked, endogenous basis.

Materials and methods. Forty hand-raised blackcaps representing equal numbers of males and females from a southern German breeding population, in which all individuals migrate annually, were kept in solitary 45 × 23 cm activity

cages. The birds had access to food ad libitum during the entire experimental period, and all conditions except photoperiod were kept constant. One group of 20 birds was kept under a photoperiod that simulated the fall migration to Mediterranean wintering grounds while the remaining 20 were held under conditions that simulated migration to the British wintering grounds. For the purpose of making male-female comparisons these two groups are lumped together. Nocturnal migratory activity (Zugunruhe) was measured by four microswitches attached to the only two perches in each cage. Activity was recorded as the number of hops per hour from 20:00 to 06:00 h nightly. Activity data were transferred from the microswitches to a relay box and finally to a custom software program on an IBM personal computer AT for storage and analysis.

Results. During the majority of the migratory period females showed, on average, higher levels of autumnal migratory activity than males (fig.). When averaged for the entire migratory period, the mean activity (± 1 SE) per bird per night was significantly higher in females relative to males (671.0 ± 185.5 hops/bird-night vs 344.5 ± 83.8 hops/bird-night, respectively) based on a two-sample t-test ($p < 0.001$). When we examined migratory activity on a weekly basis, we found that the difference between males and females was generally insignificant during the initial portions of the autumnal migratory period (approximately mid-September through October) but became significant ($p < 0.05$ as a minimum criteria for rejection of the null hypothesis) during the second half of the migratory period. Finally, as determined using Mann-Whitney tests, there was no difference in starting dates of migratory behavior between the sexes, but females showed significantly ($p < 0.05$) later termination dates (x date 26 December) than males (x date 15 December). Thus, females showed both significantly more migratory activity and a significantly longer migratory period than males. The initiation of spring migratory activity was significantly ($p < 0.01$) earlier for males (x date 8 March) than for females (x date 15 March). In addition, patterns of migratory fattening corresponded to sexual differences in migratory activity (i.e., in autumn females held fat deposits longer than males).

Discussion. These data indicate that distinct classes within an annual migrant population can differ significantly with respect to their endogenously programmed migratory behavior. Because it appears that differential migratory behavior



Weekly mean levels (± 1 SE) of migratory activity (hops per bird per night) shown by male (solid lines, $n = 20$) and female (dashed line, $n = 20$) blackcaps from southwest Germany.

is sexually linked in this population, this system appears promising for further explorations of genetical, physiological, ecological and evolutionary aspects of migratory behavior. It should be noted that qualitative sexual differences in migratory behavior have been detected in a partial migrant population of blackcaps as well. Males from southern France were significantly less likely to show any migratory behavior at all relative to females from the same population¹⁶. It seems reasonable to suggest that sexual differences in endogenously programmed migratory activity in autumn have ultimate roots in intraspecific competition during the nonreproductive season although other factors may certainly be involved. With respect to differential migratory activity in spring, males of most migrant species precede females in arriving at the breeding grounds, presumably to establish territories at the earliest possible time. In blackcaps, males precede females by about a week¹², a pattern that matches closely the differential migratory activity displayed by our experimental birds.

Results in this direction have also been found in another annual migrant, the dark-eyed junco (*Junco hyemalis*), but the tendency toward differential migratory activity between the sexes under laboratory conditions was generally insignificant¹⁷. Juncos show a pronounced bias for females to migrate farther than males¹⁷. So, in combination, the laboratory and field studies indirectly implicate a relatively strong role for environmental factors in regulating sexual differential migration in this species. Recent experiments on juncos appear to support this possibility¹⁸.

Although these results indicate that an endogenous component could, in theory, be entirely responsible for differential

migration in this population of blackcaps, they do not preclude the possibility that exogenous factors might also play a potentially important proximate role. Further experimental work in this direction is currently being undertaken.

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Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4¹

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Summary. The African tree *Combretum caffrum* (Combretaceae) has been found to contain a powerful inhibitor of tubulin polymerization (IC₅₀ 2–3 µM), the growth of murine lymphocytic leukemia (L 1210 and P 388 with ED₅₀ ~ 0.003 µM and human colon cancer cell lines [e.g. LoVo (ED₅₀ = 0.005 µg/ml), HT 29 (ED₅₀ 0.02 µg/ml, Colo 205 (ED₅₀ = 0.07 µg/ml), DLD-1 (ED₅₀ = 0.005 µg/ml) and HCT-15 (ED₅₀ = 0.0009 µg/ml)] designated combretastatin A-4 (**1c**). The structure assigned by spectral techniques was confirmed by synthesis.

Key words. Combretaceae; *Combretum caffrum*; combretastatin A-4; inhibitor tubulin; lymphocytic leukemia; human colon cancer.

The willow-like appearance of the south African tree *Combretum caffrum* (shrub to 15 m high, Combretaceae) is a common sight overhanging stream beds, and the powdered root bark is used by the Zulu as a charm to harm an enemy². Previously we reported the isolation of a series of bibenzyls³, stilbenes^{4,5}, and phenanthrenes⁶ from this very productive tree. All were found active against the murine P 388 lymphocytic leukemia cell line, and some, especially combretastatins A-1 (**1a**)⁴ and A-2 (**1b**)⁵, were shown to be potent inhibitors of microtubule assembly. We now report that a trace fraction (26.4 mg from 77 kg of dry stem wood), selected due to inhibition of the P 388 cell line and tubulin polymerization, was found to contain a powerful inhibitor of microtubule assembly (IC₅₀ 2–3 µM) named combretastatin A-4 (**1c**). The *cis*-stilbene (**1c**) was found comparable in its inhibitory effects to podophyllotoxin and combretastatin A-1 and more potent than colchicine and steganacin. An

additional new compound, combretastatin A-5 (**1d**) was also isolated from this fraction, but it was significantly less active. The 0.65 g fraction that just preceded one employed to separate fractions containing combretastatins A-3 and B-2⁵ (during partition chromatography on Sephadex LH-20 in 3:1:1 hexane-toluene-methanol) was further refined by a series of liquid (3:1 and 4:1 hexane-ethyl acetate) chromatographic steps on silica gel and by HPLC (Partisil M-9, 9:1 hexane-2-propanol). While the resulting P 388 active fraction (26.4 mg) at first appeared homogeneous, high field (400 MHz) ¹H-NMR suggested a mixture of at least three compounds that resisted separation until conversion (7.1 mg) to *t*-buthyldimethylsilyl ether derivatives (cf., **1e** and **1f**). Multiple development preparative thin layer chromatography on silica gel in 17:3 hexane-ethyl acetate afforded silyl ethers **1e** (3.0 mg, oil) and **1f** (2.0 mg, oil) corresponding, respectively, to combretastatins A-4 and A-5. The