

THE EVOLUTIONARY SIGNIFICANCE OF DEVELOPMENTAL PLASTICITY IN
THE COMMUNICATION SYSTEM OF *NEOCONOCEPHALUS TRIOPS*
(ORTHOPTERA: TETTIGONIIDAE)

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by
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THE EVOLUTIONARY SIGNIFICANCE OF DEVELOPMENTAL PLASTICITY IN
THE COMMUNICATION SYSTEM OF *NEOCONOCEPHALUS TRIOPS*
(ORTHOPTERA: TETTIGONIIDAE)

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ABSTRACT

The katydid *Neoconocephalus triops* is a tropical species that extended its distribution range to North America. In North America, males display developmental plasticity in mating calls between reproductive generations: the winter call differs from the summer call in both double-pulse rate and call structure. In the tropics, males express only the summer call.

We found that tropical *N. triops* have the capacity to express the winter call, but tropical conditions do not induce its expression. Female *N. triops* have strong preferences for double-pulse rate, but are not selective for call structure, i.e. females of most populations are attracted to both the summer and winter call structure. The temperature dependency of female preference for double-pulse rate in the Florida population was significantly steeper than those of tropical populations or other *Neoconocephalus* species. This steeper temperature dependency of female preference resulted in an overlap of the preference with the summer call at high temperatures, and the winter call at low temperatures, facilitating communication in both seasons. Thus, female preference evolved in response to male call plasticity in the Florida population of *N. triops*.

Chapter 1

Developmental plasticity, communication, and the study system:

Neoconocephalus triops

Developmental plasticity

One fundamental question of biology is, how did the diversity of traits and species that we observe today evolve? Recent research indicates developmental (or phenotypic) plasticity to be a potent source of diversification (Roth, 1992; Emlen, 1994, 1997b; review in West-Eberhard, 2003). Studying how developmental plasticity leads to the diversification of traits is therefore an important contribution to the understanding of evolutionary processes.

The phenotype of an organism is the product of both the genotype and environment. The phenotype is characterized by its flexibility and responsiveness to environmental cues, traits that are summarized in the term developmental plasticity (review in West-Eberhard, 2003). Environmental cues can be mediated through the internal (e.g. hormones) or external (i.e. abiotic and biotic) environment of an organism. The response of the phenotype to these cues can be diverse, including reversible, irreversible, adaptive, non-adaptive, as well as continuous and discontinuous changes (West-Eberhard 2003).

There are two main reasons why environmentally induced traits are considered to have a high evolutionary potential. First, environmentally induced traits can be immediately widespread. A new environmental factor can affect many individuals within a single generation at once. Thus, the environmentally induced traits have superior

chances of occurrence in a favorable genetic background. That is, the more individuals are affected, the higher the chances are of the induced trait to occur in a genetic or phenotypic background that can "utilize" the induced change (West-Eberhard 2005). Second, there is a high probability of initial matching of environmentally induced traits with the conditions in which they are selected. The environmentally induced trait is strongly associated, at least part of the time, with the particular environmental situation that induced it. Thus, environmentally induced traits are rendered more subject to consistent selection and directional modification (West-Eberhard 2003).

Developmental plasticity as initiator of evolutionary change

Developmental plasticity can lead to evolutionary change in different ways. First, a change in environmental conditions can lead to phenotypic change within a population due to the flexibility of phenotypes. Since there is variability between individuals in the ability to respond to these environmental cues, the new environmental input will generate phenotypic variation among individuals. Selection will act on the phenotypic variation if this variation has a fitness effect, and will lead to evolutionary genetic change if the phenotypic variation has a genetic component (West-Eberhard 2003). Thus, the environment can induce phenotypic variation due to developmental plasticity and subsequent selection may cause genetic change.

Second, developmental plasticity itself is a trait with a partial genetic basis that can evolve (West-Eberhard 1989). Either a change of the environmental conditions in the current habitat or a change of environmental conditions due to spread into a new habitat can lead to a change of selective pressures that act on developmental plasticity. As a

result, developmental plasticity can be lost, gained, or changed in the degree of its expression.

A gain of developmental plasticity is likely if the new environmental conditions require the expression of more than one phenotype. For example, it may be crucial to express alternative phenotypes to survive in seasonal environments (Shapiro 1976, Beebe 1949, Haber 1993, Dingle 1996) or in environments that exhibit extreme conditions in an unpredictable manner (Laurila et al. 2002, Trussell & Smith 2000, Pfennig & Murphy 2000). In these cases, developmental plasticity changed qualitatively, i.e. the developmental capacity to express more than one phenotype increased.

If the new environmental conditions only exhibit part of the variation of the original environment (e.g. seasonal versus non-seasonal), one phenotypic alternative might be better suited and thus expressed in these conditions. If only one of the alternative phenotypes is expressed in a given environment, the other 'hidden' phenotype might be lost due to genetic drift or selection (Masaki, 1986; Pigliucci et al. 2006; West-Eberhard 1989, Pfennig & Murphy 2000). The fixation of the one phenotype thus results in a reduction of the capacity of developmental plasticity. If the expression of a single phenotype becomes genetically fixed, its expression will become independent of environmental factors (= *genetic assimilation*, Waddington 1961). Note that the expression of a single phenotype can also become environmentally fixed, i.e. only one phenotype may be expressed because the environmental conditions inducing it remain constant (West-Eberhard 2003).

If the new environment is more or less diverse than the original environment, it is also possible that the alternative phenotypes can change quantitatively from each other.

That is, only the degree in which alternative phenotypes differ from each other changes rather than the number of alternative phenotypes expressed.

Third, the environmentally induced variation of a trait (adaptive or non-adaptive) will cause the evolution of a related trait to compensate for the induced change. My study represents an example of this case.

Acoustic communication and temperature

Potent systems to study the evolutionary role of developmental plasticity are communication systems. Since reproductive communication directly influences gene flow, changes in the communication system can lead to rapid evolutionary change and speciation. In most acoustic communication systems males produce mating calls and females approach the calling males for mating (Gerhardt & Huber 2002). The call contains temporal and/or spectral elements that are specific for each species and these elements serve for species identification and mate attraction (Deily & Schul 2004, 2006). A match between male call properties and female preference for these parameters is crucial for communication systems to function.

Ambient temperature is a very potent environmental factor that influences communication of poikilothermic animals. Temperature can cause changes of temporal parameters (e.g. pulse rate) of acoustic signals (Gayou, 1984; Walker, 1957, 1975, 2000) as well as properties of female preference (Walker 1957, Gerhardt 1978, Pires & Hoy 1992). Since other physiological elements are involved in sound production (muscles and neurons) than in sound perception/processing (neurons only, Gerhard & Huber 2002), temperature dependencies of sender and receiver inherently differ from each other. As a

result, calls and call recognition are usually not well matched throughout a broad range of temperatures. Anurans and insects solve this 'temperature problem' in different ways. In some species, temperature dependencies of call and call recognition are rather similar, resulting in a match between sender and receiver at biologically relevant temperatures (e.g. Gerhardt, 1978, Walker, 1957, 1975, 2000; Doherty, 1985). Other species employ temperature-invariable call parameters such as ratios or duty cycles in order to identify mates (e.g. Helversen, 1972; Helversen 1979). In the grasshopper *Chortippus biguttulus*, female preference is tuned rather broadly to cover the range of temperature related variation of the call (Helversen & Helversen, 1981). Note that we consider the temperature dependency of call and call recognition as (physiological) traits of the communication system rather than cases of developmental plasticity of sender and receiver.

There are only a few documented cases of developmental plasticity in communication systems. In crickets, temperature caused a change of pulse rate of the male calls: Males reared (Walker 2000) or housed (Grace & Shaw 2004) at low temperatures produced calls with slower pulse rates than males reared at higher temperatures. In the cricket *Laupala cerasina*, the change of pulse rate could be reversed by housing males for an extended period of time under high (or low) temperatures. In the same species, female preference also responded to temperature: females reared to adulthood at high temperatures preferred the higher pulse rates than females that were reared at lower temperatures (Grace & Shaw 2004). It is not clear, however, if these changes in female preference are reversible like the changes in the male calls.

In the katydid *Neoconocephalus triops*, the environment induces differences in its mating call of such great magnitude that it was originally described as separate species (Whitesell 1974). The degree of the expressed developmental plasticity, the exceptional distribution range of the species, and the simplicity of the conditions inducing different phenotypes identify *N. triops* as model system to investigate the role of developmental plasticity in evolution of phenotypes.

The study system: *Neoconocephalus triops*

The distribution of *N. triops* ranges from South America (Argentina, Systax data base, University of Ulm-Germany) throughout Central America to temperate North America (Ohio, Whitesell, 1974). Within North America, *N. triops* spreads from east coast to west coast (Whitesell, 1974, Fig. 1). Direct egg development and adult diapause suggest a tropical origin of *N. triops* and a subsequent spread into temperate North America (Whitesell, 1974).

In the southern part of North America, populations have one reproductive generation in the summer and one in the winter (Fig. 2). However, a given individual reproduces either in the summer or in the winter and not in both generations (Whitesell 1974). Offspring of the summer generation diapause as adults and reproduce at the end of the following winter/early spring (= winter generation). Offspring of the winter generation form the following summer generation. Offspring of the winter generation that mature too late in the season skip the summer and become reproductively active in the following winter (Whitesell, 1974). Thus, the winter generation contains genes of the whole population, whereas the summer generation contains only part of the gene pool

(Whitesell 1974). In the northernmost part of the distribution range (e.g. Ohio), populations reproduce only once per year in late spring (Fig. 3), whereas in the tropics, reproduction takes place all year (Whitesell 1974).

In populations that have a summer and a winter generation, male calls differ substantially in two call parameters between generations. First, at the same temperature, summer calls have a significantly higher double-pulse rate (>20%) than winter calls (Fig. 3). Second, calls of summer males (= summer calls) are structured in verses whereas calls of winter males (= winter calls) are continuous. These differences of the calls are induced by the day-length experienced during juvenile development (Whitesell & Walker, 1978). The expression of these two call phenotypes differs throughout the distribution range of *N. triops*. Northernmost populations (e.g. Ohio) only express the winter call phenotype (Whitesell 1974) and tropical populations express only the summer call phenotype in their natural environment (Greenfield 1990).

For my dissertation, I investigated evolutionary role of developmental plasticity in the communication system of *N. triops*. In the second chapter, I determined in the population from Florida how female preference responded to the seasonally expressed developmental plasticity of male calls. In the third chapter, I investigated whether males from tropical populations have the capacity for call plasticity. In the fourth chapter, I compared the temperature dependency of female preference between populations that express call plasticity (North America) and populations that do not express call plasticity (tropics). In the last chapter, I summarize the results and discuss the role of the environment and developmental plasticity for evolutionary processes.

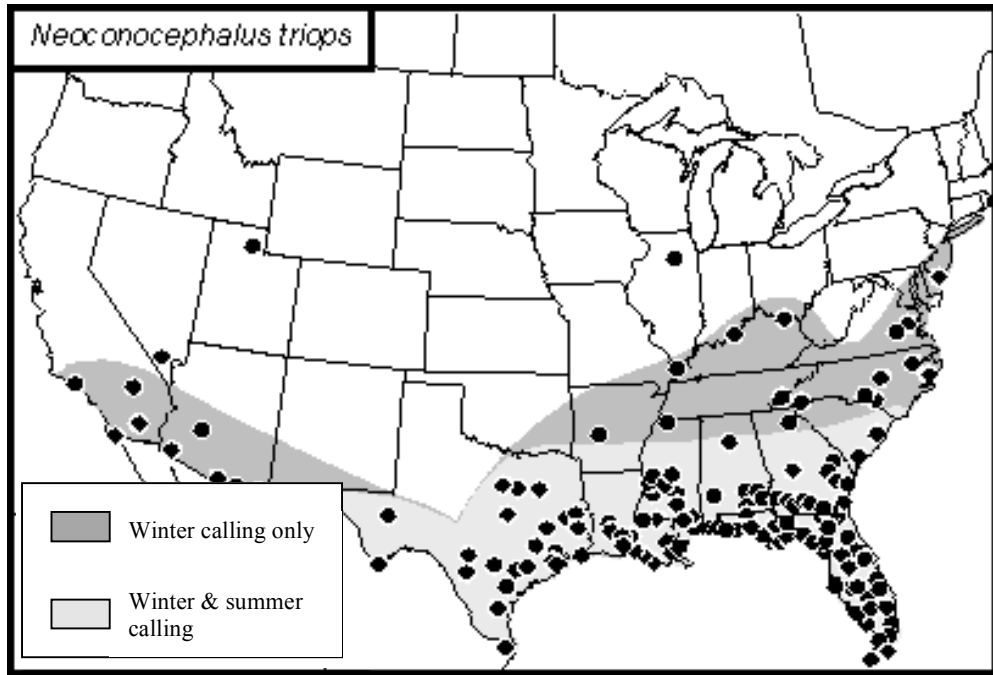


Fig. 1

Distribution of *N. triops* in North America. Light gray areas indicate spring and summer singing (i.e. winter and summer generations), dark gray areas indicate only spring singing (i.e. only winter generation; Figure from Walker 2001).

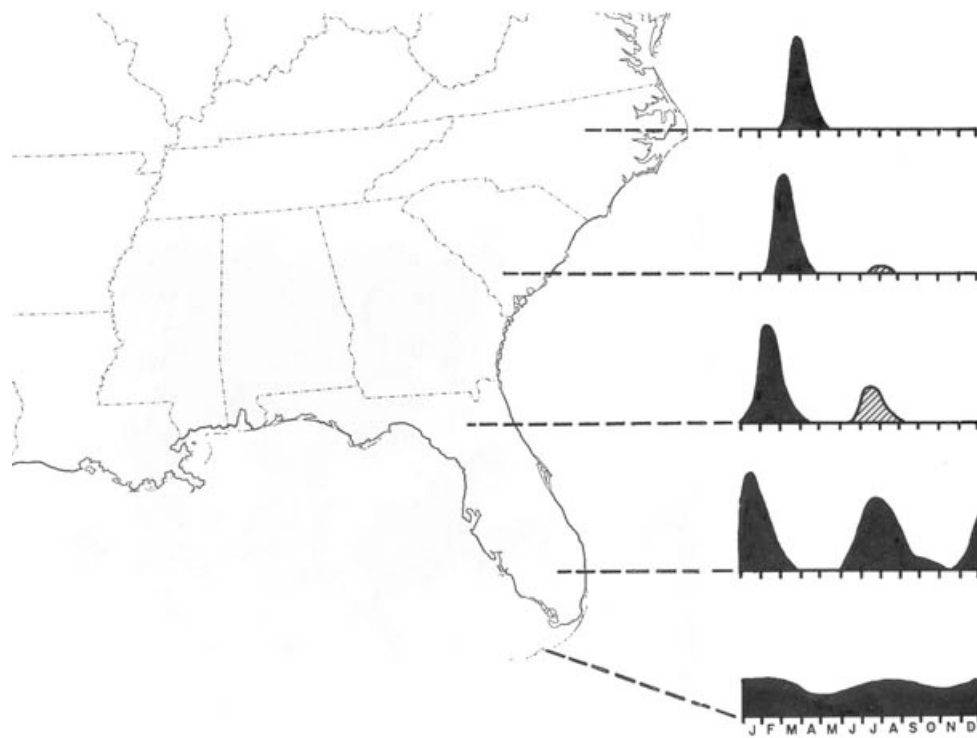


Fig. 2

Geographic variation of life cycles of *N. triops*. In the northernmost distribution range, *N. triops* has only one spring generation and produces only the winter call phenotype. From South Carolina to northern Florida, *N. triops* has a second generation in the summer that expresses the summer call phenotype. From the Florida Keys through out the Caribbean, *N. triops* reproduces continuously throughout the year and produces only the summer call phenotype (figure from Walker 2001).

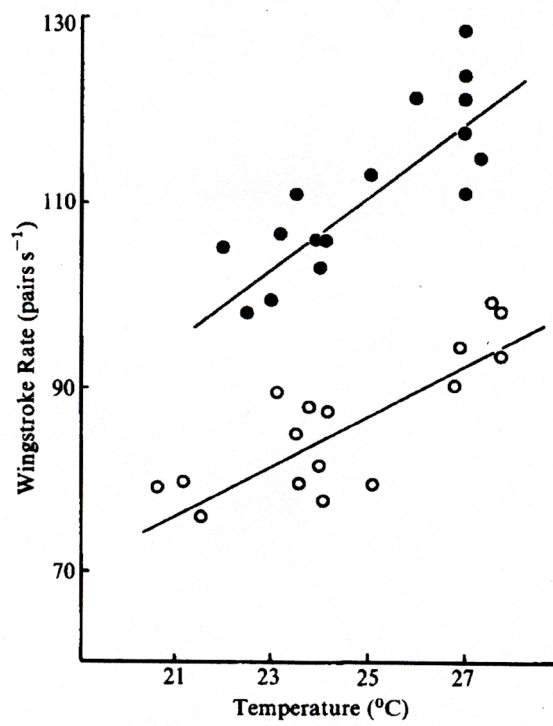


Fig. 3

Wingstroke rates (= double-pulse rates) of summer and winter males from northern Florida. Filled circles indicate wingstroke rates of summer males, open circles indicate that of winter males (figure from Whitesell & Walker 1978).

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Chapter 2

Developmental plasticity of mating calls enables acoustic communication in diverse environments

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Abstract

Male calls of the katydid *Neoconocephalus triops* exhibit substantial developmental plasticity in two parameters: (1) Calls of winter males are continuous and lack the verse structure of summer calls. (2) At equal temperatures, summer males produce calls with a substantially higher pulse rate than winter males. We raised female *N. triops* under conditions that reliably induced either summer or winter phenotype and tested their preferences for the call parameters that differ between summer and winter males.

Neither generation was selective for the presence of verses, but females had strong preferences for pulse rates: only a narrow range of pulse rates was attractive. The attractive ranges did not differ between summer and winter females. Both male pulse rate of calls and female preference for pulse rate changed with ambient temperature, but female preference changed more than male calls.

As a result, the summer call was only attractive at 25°C, whereas the slower winter call was only attractive at 20°C. Thus, developmental plasticity of male calls compensates for differences in temperature dependency between calls and preference and enables the communication system to function in heterogeneous environments. The potential role of call plasticity during the invasion of new habitats is discussed.

Keywords: Phenotypic plasticity, *Neoconocephalus triops*, phonotaxis, temperature dependency, communication

Introduction

Phenotypes are often strongly influenced by the environment during development. Identical genotypes may result in significantly different phenotypes depending on environmental conditions (West-Eberhard 2003). The potential of such developmental (or phenotypic) plasticity has recently been recognized, as a mechanism for the evolution of novel traits (review in West-Eberhard 2003). Furthermore, developmental plasticity may be crucial for living in variable environments (seasonal and non-seasonal, Shapiro 1976; Cook & Johnson 1968; Trussel & Smith 2000; Agrawal 2001) and may facilitate the spread of a species into new environments (Agrawal 2001; Yeh & Price 2004; Losos et al. 2000).

One important function of acoustic communication in insects and anurans is to identify conspecific mates. Accordingly, call parameters used by the receiver to identify conspecific signals exhibit little within-male variation and between-male variation (Gerhardt 1991; Gerhardt & Huber 2002). This situation is complicated by the temperature dependency of many call parameters (e.g. pulse rate), which increase or decrease in value with changing ambient temperatures. In case of temperature dependent parameters used for species identification, the mean value changes with temperature, while variation at any given temperature remains small (Gerhardt & Huber 2002). Correct species identification is then assured, by matching the temperature dependency of

the receiver (= call recognizer) to that of the sender throughout the relevant temperature range (Gerhardt 1978; Helversen & Helversen 1981; Doherty 1985).

In the katydid *Neoconocephalus triops* (Orthoptera: Tettigoniidae) females identify and approach calling males for mating. In North America, this species occurs as two seasonally distinct generations, one in the summer and one in the winter (Whitesell 1974; Whitesell & Walker 1978). In general, the offspring of one generation produce the next generation, so that both generations constitute one gene pool (Whitesell 1978). For a detailed description of natural history of *N. triops* see methods.

Male calls of *N. triops* are significantly influenced by the photoperiod experienced during juvenile development (Whitesell & Walker 1978). This developmental plasticity results in striking differences between the calls of the two generations. First, winter males produce continuous calls, whereas the calls of summer males are structured in verses (Whitesell & Walker 1978; Greenfield 1990). Second, at equal ambient temperature, the average double pulse rate in calls of summer males was more than 20% higher than that of winter males with low between male variation within each generation (Coefficient of Variation: 6.02 - 6.72%, Fig. 1). In fact, the call differences are so striking, that seasonal generations of *N. triops* were originally considered separate species (Walker 1964; Whitesell 1974).

Here we investigate the consequences of the plasticity of male calls for the communication system of *N. triops*. Females were raised under environmental conditions that induce the different call phenotypes. We tested female preferences of both generations for the call parameters that differ between summer and winter generations. Female preferences did not differ between generations, i.e. they did not exhibit

developmental plasticity that parallels the plasticity of male calls. Rather, male call plasticity enables communication in seasonally variable environments.

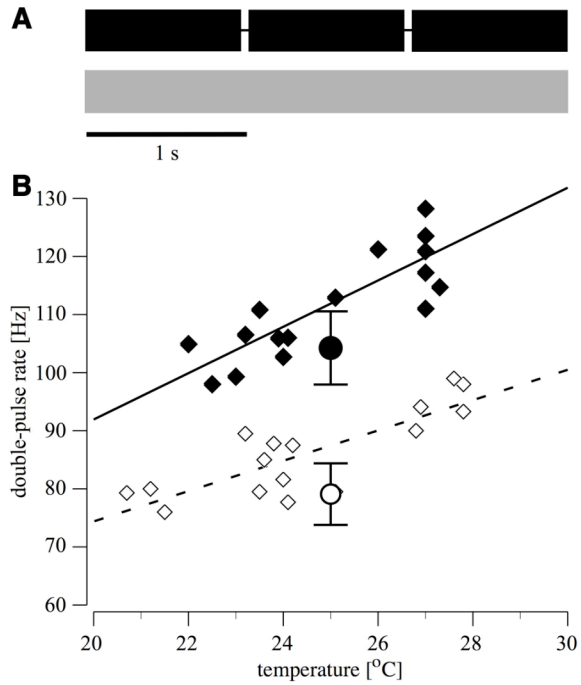


Fig. 1

Developmental plasticity of male calls of *Neoconocephalus triops*. (A) Schematic call structures of versed summer call (upper trace) and continuous winter call (lower trace).

(B) Temperature dependencies of summer and winter calls. Black diamonds indicate amplitude modulation (AM)-rates (double-pulse rates) of summer calls, open diamonds indicate rates of winter calls (data and regression lines from Whitesell & Walker 1978). Black circle indicates mean AM rate (\pm SD) of calls of summer males ($n = 25$) raised in this study, and the open circle indicates that of raised winter males ($N = 15$).

Material and Methods

Natural history of N. triops

The distribution of *N. triops* ranges from the tropics in South America to the temperate regions of North America. In North America, two distinct reproductive generations occur per year. One generation reproduces during the summer and the other generation reproduces in late winter/early spring (Whitesell & Walker 1978). The two generations do not form distinct gene pools, as each generation is largely comprised of the offspring of the previous generation (Whitesell 1974). The offspring of the summer generation diapause as adults during the coldest months of the year before they reproduce in the late winter. Offspring of the winter generation mature without diapause and reproduce in the following summer. Males of these populations express the call plasticity described above. In the tropics, reproductively active individuals of *N. triops* can be found all year round. Males of these populations express only the summer call (Greenfield 1990; Whitesell 1974).

Rearing of animals

Adult *N. triops* were collected in Gainesville, Florida in winter and summer of 2004 and brought to the laboratory at the University of Missouri for egg collection. Males and females were housed on grass, which females used for oviposition. We collected eggs from the grass and stored them in petri dishes on moist filter paper. We sprayed the eggs with 0.5% Methylparaben solution to inhibit growth of fungi. Eggs were kept in the dark at 20-30°C until eclosion.

All insects were raised on potted wheat seedlings and fed with puppy chow (Purina, MO), rolled oats, and apples. Cages were kept in incubators at 50-70% relative humidity and with daily temperature and light cycles. The high temperature was maintained from one hour after lights came on until one hour before dark, during which the temperature was reduced. The specific temperature and light cycles are given below.

The phenotypic variability of male calls in *N. triops* is induced by the photoperiod experienced during juvenile development (Whitesell & Walker 1978; Whitesell 1974). A light/dark cycle of 15/9 h results in summer calls (discontinuous calls, fast double-pulse rate), whereas a cycle of 11/13h results in continuous winter calls with slow double-pulse rate (Whitesell & Walker 1978).

After eclosion, we assigned first-instar nymphs randomly to summer or winter rearing conditions (Whitesell 1974). Under summer conditions, animals were reared to adulthood with a light/dark cycle of 15/9 h and high/low temperatures of 30/20°C. We refer to these animals as ‘summer generation’ or ‘summer animals’. Male calls were recorded two to three weeks after the final molt. Females were tested in phonotaxis experiments starting three to four weeks after the final molt. Some summer females used in phonotaxis experiments were not raised from eggs, but collected as nymphs in the field and raised to adulthood in the conditions described above. We could not detect any differences in preferences between females raised from eggs and those collected as nymphs.

Under winter conditions, animals were reared to adulthood at a light/dark cycle of 11/13h and high/low temperatures of 23/17°C. Two to three weeks after adult molt, we transferred the animals to a light/dark cycle of 9.5/14.5 h and temperatures of 18/12°C to

secure diapause. After 3.5-5 months in diapause, insects were transferred to a light/dark cycle of 13/11 h and high/low temperatures of 26/18°C in order to terminate diapause. We refer to these animals as 'winter generation' or 'winter animals.' Male calls were recorded one to three weeks after termination of diapause. Females were tested in phonotaxis experiments starting three to four weeks after the end of diapause. The photoperiods and temperatures used in this study correspond approximately to the natural conditions each generation experiences in Gainesville, FL (www.wunderground.com).

We recorded calls of males raised under both summer and winter conditions (for details of call recording and analysis see Schul & Patterson 2003). Calls of the summer generation were structured in verses, whereas winter males produced continuous calls; Whitesell & Walker (1978) reported the same pattern for summer and winter calls. Calls of reared summer males had a significantly higher double-pulse rate (mean \pm SD at 25°C: 104.3 ± 6.3 Hz, $n = 25$) than calls of reared winter males (79.1 ± 5.3 , $n = 15$; T-test, $p < 0.001$, see Fig. 1). Whitesell and Walker (1978) reported double-pulse rates at 25°C in the range of 103-112 Hz and 78-88 Hz for summer and winter calls, respectively. Thus, our rearing conditions reliably resulted in the call phenotypes found in summer and winter animals.

Female phonotaxis tests

We tested female phonotaxis using a walking compensator (Kramer-Kugel, Weber et al. 1981) in a temperature-regulated anechoic chamber at ambient temperatures of $20 \pm 1^\circ\text{C}$ and $25 \pm 1^\circ\text{C}$. In short, females were placed on top of a sphere, free to walk but kept in place by compensatory sphere rotations, while acoustic signals were presented

from loudspeakers located in the animal's horizontal plane. The intended direction and speed of the animal were read from the control circuitry. All experiments were performed in the dark except for the infrared light used to monitor the insects' position. (For details see Schul 1998; Weber et al. 1981). Females were tested for as long as 6 weeks; we did not detect any change in preference during this period.

Stimulation

We generated synthetic signals using a custom-developed DA-converter/amplifier system with 250 kHz sampling rate and 16-bit resolution. We delivered the stimuli using one of two loudspeakers (Motorola KSN1218C) mounted at a distance of 150 cm in the horizontal plane of the animal and separated by an angle of 105°. Signal amplitude was set to 80 ± 1 dB peak SPL (re. 20×10^{-5} Pa) using a Bruel and Kjaer sound level meter (B&K 2231) and a ¼" condenser microphone (G.R.A.S 40BF) which was positioned 1 cm above the sphere.

Most of the energy in the calls of *N. triops* is concentrated in a low frequency band centered around 11 kHz, and frequency components in the ultrasound are at least 20 dB less intense than the low frequency band. We used a pure tone of 11 kHz as a carrier signal to which we subsequently applied amplitude modulations.

We based temporal parameters of our stimuli on our analysis of calls from natural populations. The control stimulus of the 25°C experiments consisted of a train of paired pulses of 2 ms and 2.75 ms duration with an interval of 1.75 ms in between. These double-pulses were repeated after an interval of 2.5 ms, resulting in a double-pulse rate of 111 Hz. For the control stimulus in 20°C experiments, we used pulse durations of 3 ms

and 3.2 ms and interval durations of 2.8 ms and 4.4 ms, resulting in a double-pulse rate of 75 Hz. The amplitude of the first pulse of the pair was 50% of that of the second pulse in both control stimuli. In experiments with summer animals, the control stimulus was structured in verses with durations of 950 ms separated by silent intervals of 50 ms. In experiments with winter animals, the control stimulus was continuous. For both winter and summer females, responses to the control stimulus did not differ significantly in response strength from responses to natural calls.

We determined in preliminary tests the relevant call parameters for this study (data shown in appendix). We found that female *N. triops* did not require the elaborate double-pulse structure of the male call: stimuli with the double-pulse replaced by one long pulse of the duration of the double-pulse (= merged pulses) were as attractive as stimuli with the double-pulse pattern (see Deily & Schul 2004; Schul 1998 for similar results in other katydid species). This result indicates that the relevant amplitude modulation (AM) of *N. triops* calls is the rate at which double-pulses are repeated. In the tests presented in this study, we therefore use such merged pulses (equivalent to double-pulses in natural calls). We refer to the rate of these merged pulses and to the equivalent double-pulse rate in natural calls as AM-rate.

In the first set of tests we determined female preference for call structure (Fig. 2). We used stimuli (AM-rate of 110 Hz) that were either continuous (winter call structure) or structured in verses with durations of 950 ms and intervals of 50 ms (summer call structure). We compared responses of summer and winter generations at an ambient temperature of 25°C.

The second set of tests examined female preference for AM-rate (Fig. 3) at two ambient temperatures (20°C and 25°C). All stimuli had a duty cycle of 72% (duty cycle = pulse duration/pulse period). AM-rates were varied between 55 and 160 Hz. In preliminary experiments we confirmed that AM-rate rather than pulse duration or interval duration was the critical parameter evaluated by female *N. triops* (see appendix).

Experimental protocol

Each stimulus tested in this study was presented for 60-90 s from each of two different loudspeaker positions. The first stimulus of each test series was the control stimulus, followed by two to three test stimuli, another control, etc. A period of 60 s of silence was given between stimuli. The experimental series lasted between 30 and 60 min and consisted of up to six test stimuli (plus up to four controls). We randomly varied the sequence of stimuli within a series among individual females. (For a detailed description of the experimental protocol see Schul 1998; Bush et al. 2002).

Data analysis

We quantified female response to a given stimulus by calculating a phonotaxis score (Schul 1998). The phonotaxis score included three measures describing the relative strength of phonotaxis: (1) the walking speed relative to the walking speed during the control stimulus, describing the elicited locomotion activity; (2) the vector length, describing the accuracy of orientation; and (3) the angular orientation relative to that during the control stimulus. The score can range from approximately +1 to -1 representing perfect positive or negative phonotaxis, respectively. Phonotaxis scores close to 0 indicate random orientation or no response (for details see Schul 1998).

In the first experiment we compared female responses to continuous calls and versed calls (Fig. 2) using Wilcoxon-signed rank tests (Zar 1984). To compare female preference for AM-rate between summer and winter generation in the second experiment (Fig. 3), we plotted phonotaxis scores as a function of AM-rate. These response functions were smoothed by calculating a gliding average through three values and then normalizing. We determined upper and lower cutoff rates by fitting a parabolic function ($y = c - a * (x-b)^2$) to each individual's response function by minimizing the sum of squared errors. Upper and lower cutoff rates were defined as the AM-rate where the fitted function fell below 70%. We compared the upper and lower cutoffs between generations at the same temperature using Mann-Whitney U-tests (Zar 1984). The application of cutoff frequencies in our study is only meant to emphasize the relative attractiveness of stimuli and do not classify stimuli as 'recognized' or 'not recognized' (for a detailed discussion see Bush et al. 2002). Unless otherwise stated, data are given as mean \pm standard error of the mean (SEM).

Results

First, we tested the preference of summer and winter females for the call structure (Fig.2). For this parameter, we could not detect differences in female preference between summer and winter generations. In both generations responses were high to the continuous call model (= winter call) and to the call model with verse structure (= summer call). Continuous calls elicited slightly higher scores than did the versed calls (summer females: 0.95 ± 0.04 vs. 0.84 ± 0.03 , mean \pm sem, $n = 11$; winter females: 0.94

± 0.05 and 0.84 ± 0.05 , $n = 10$). These differences were marginally significant for both generations (Wilcoxon signed-rank test, $0.1 \geq p > 0.05$).

In the second set of experiments, we compared the preference function for AM-rate between summer and winter females at two ambient temperatures (20°C and 25°C). At 20°C, phonotaxis scores of both generations were highest around 75 Hz and decreased towards higher and lower AM-rates (Fig. 3 a, b). The lower and upper cutoff frequencies did not differ significantly between the summer and winter generation (lower cutoff: summer 67.4 ± 1.09 Hz, winter 64.7 ± 0.47 Hz; upper cutoff: summer 88.9 ± 1.23 Hz, winter 86.4 ± 1.65 Hz; $n = 11$ (summer) or 7 (winter); Mann-Whitney U-test, all tests $p > 0.05$).

At 25°C, females of both generations responded strongest to AM-rates close to 105 Hz; higher and lower AM-rates elicited weaker responses (Fig. 3 a, b). As at 20°C, lower and upper cutoff frequencies did not differ significantly between generations (lower cutoff: summer 95.4 ± 1.45 Hz, winter 89.6 ± 3.01 Hz; upper cutoff: summer 121.3 ± 1.96 Hz, winter 116.7 ± 1.96 Hz; $n = 10$ (summer) or 9 (winter); Mann-Whitney U-test, all tests $p > 0.05$). Thus, we could not detect any differences between the two generations in their preference for AM-rates.

Comparing female preferences for AM-rate with the AM-rate of male calls demonstrates distinct mismatches at some temperatures. At 20°C, preference of both summer and winter females was tuned to the AM-rate of the winter calls at this temperature. However, the model of the summer call at 20°C evoked only weak responses ($PS < 0.5$, Fig. 3) in females of either generation. Conversely, at 25°C preference of both summer and winter females was tuned to the AM-rate of the summer call, while the AM-

rate of the winter call at the same temperature was much less attractive ($PS < 0.4$, Fig. 3). Thus, male calls and female preference were well matched only at temperatures corresponding to the season when each generation calls: at 25°C only the summer call was attractive, while at 20°C only the winter call elicited strong responses.

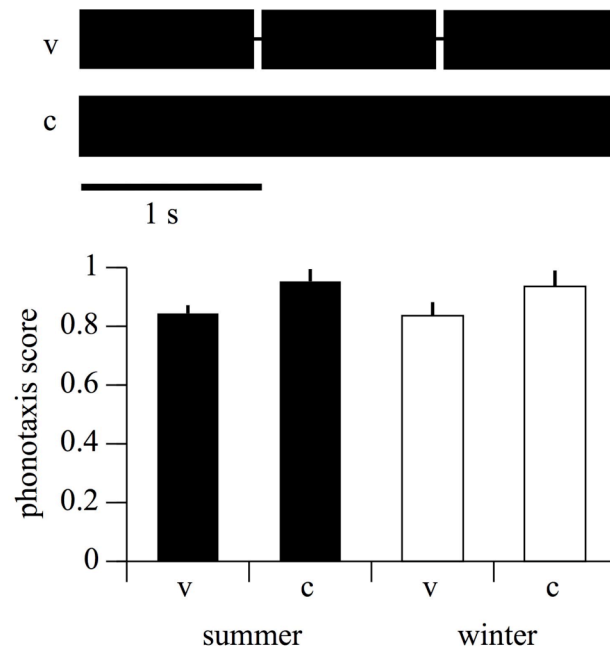


Fig. 2

Female preference to the verse structure of male calls in *N. triops*. (Top) Schematic representation of tested call model with verse structure (v, verse duration: 950 ms; interval duration: 50 ms), and continuous call model (c). (Bottom) Mean phonotaxis scores (\pm SEM) of summer females (n = 11; black bars) and winter females (n = 10; open bars) to versed call models (v) and continuous call models (c).

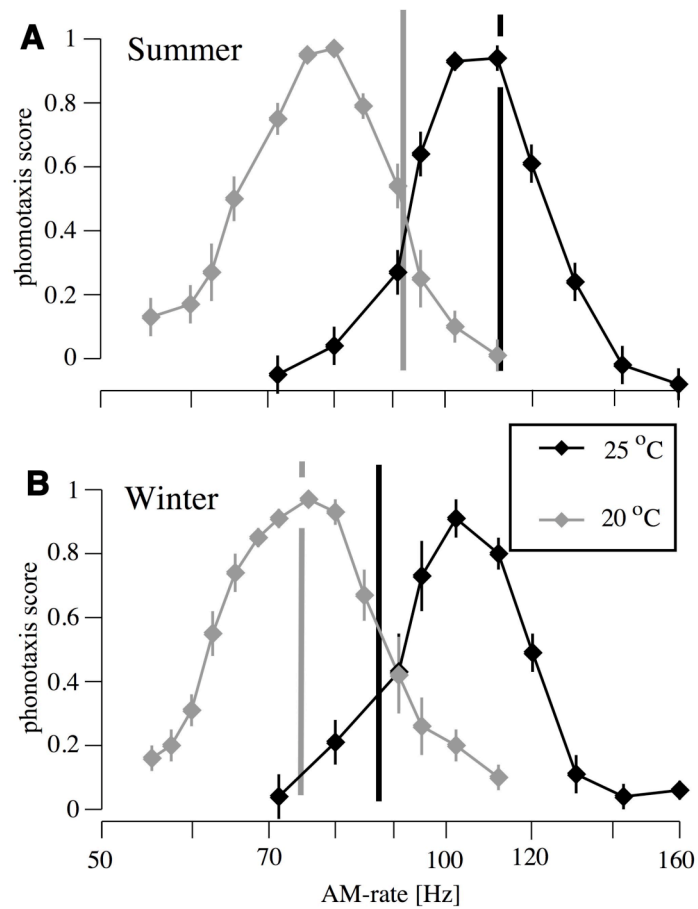


Fig. 3

Preference for AM-rate in summer females (A) and winter females (B) at 20°C (gray) and 25°C ambient temperature (black). Each curve gives the mean (\pm SEM) of normalized phonotaxis scores (summer females 20°C: $n = 11$, 25°C: $n = 10$; winter females 20°C: $n = 7$, 25°C: $n = 9$). Vertical lines indicate AM-rates (from Whitesell & Walker 1978) of male calls at 20°C (gray, left) and 25°C (black, right) for each generation.

Discussion

The calls of the summer and winter generation of *N. triops* differ in two temporal aspects. First, summer calls are structured in verses while winter calls are continuous (Fig. 1). Second, summer calls have a substantially higher AM-rate than winter calls at equal temperatures. In contrast, there were no differences in female preference between summer and winter generations: females of both generations responded readily to calls with and without verse structure (Fig. 2) and we could not detect differences in their tuning for AM-rate (Fig. 3).

The presence or absence of a verse structure in the calls had little influence on their attractiveness: both models were highly attractive to females of both generations (Fig. 2). Thus, this call characteristic is most likely not relevant for the attraction of females. A potential function of the verse pattern of the summer calls could be to provide males with silent windows during which they can detect the calls of other signalers (Greenfield 1990). The selective disadvantage of the absence of such a detection window in winter calls is likely to be small, because population densities are lower during the winter, and no calling congeners are present (Whitesell 1974).

In several cricket species, pulse rate of male calls was influenced by the temperature during development, in that the pulse rates of males raised at lower temperatures were 5 - 10% lower when recorded at the same temperature (e.g. Walker 2000; Grace & Shaw 2004). In one species (*Laupala cerasina*), female preferences changed accordingly with temperature during development. Females raised at low temperatures preferred lower rates than females raised at higher temperatures (Grace &

Shaw 2004). In contrast, alternative call phenotypes in *N. triops* were induced by the photoperiod during development and not the temperature (Whitesell 1974). Also, in *N. triops*, developmental plasticity was limited to male calls, while female preferences were not affected by photoperiod or temperature during development.

In *N. triops*, the developmental plasticity of AM-rates results in a match between the calls of the summer males and female preference at high temperatures and the winter call and female preference at low temperatures. Therefore, we argue that developmental plasticity of AM-rates is most likely a mechanism to match the temperature dependencies of calls and female preference at substantially different temperature ranges.

Female preference for AM-rates changed with temperature, as is expected for poikilothermic animals (Randall et al. 1997); an increase in ambient temperature of 10°C results in approximately a doubling of the most attractive AM-rate (Fig. 4). The temperature dependency of the AM-rate of male calls, however, is considerably less steep than that of female preferences (Fig. 4). Because of the exceptionally fast AM-rates in *Neoconocephalus* (Greenfield 1990), the muscular activity during calling produces considerable amounts of heat, elevating thorax temperature by up to 15°C above ambient temperature (Heath & Josephson 1970; Josephson 1984). The increase in thoracic temperature is larger for lower ambient temperatures, so that thorax temperature during calling is almost constant (Heath & Josephson 1970), resulting in only a weak dependence of male AM-rates on ambient temperature (Walker 1975).

Because male calls and female preferences differ in their temperature dependency, male AM-rates and female preferences are matched only in a narrow temperature range in *N. triops*. For populations in the tropics, which express only the

summer call (Greenfield 1990), this narrow temperature range appears sufficient, as ambient temperatures fluctuate little throughout the year. Moreover, because *N. triops* calls after sunset, the influence of radiant heat is negligible.

Temperate populations of *N. triops* that encounter distinct seasons overwinter as adults. Their eggs develop directly without diapause (Whitesell 1974), allowing for two distinct generations per year, one reproducing in the summer, the other in late winter/early spring. Since summer and winter generations experience different ambient temperatures, the narrow range of temperature matching between calls and call preference seems to be insufficient: summer calls were attractive only at temperatures typical for their season (approx. 25-28°C, Fig. 4); at temperatures encountered during late winter and early spring (approx. 16-20°C, Fig. 4) females responded only weakly to summer calls. Conversely, winter calls were only attractive at low, but not high temperatures. The developmental plasticity of the mating call solves this problem by allowing two narrow ranges of temperature matching, one optimized for summer temperatures and the other for winter temperatures (Fig. 4).

Whether call plasticity in *N. triops* originated in a temperate or tropical population remains unclear. Call plasticity could have evolved as an adaptation to the seasonal temperatures encountered by populations in North America. Alternatively, call plasticity could have been a latent trait shared among all populations but only induced in those that live in a suitable environment. In either case, call plasticity most likely explains today's ecological success of *N. triops*. This species has the most extensive distribution range of any *Neoconocephalus* species (Walker & Greenfield 1983) and is the only member of the genus to inhabit both tropical and temperate regions (Greenfield 1990). All other species

are confined to one region or the other. Developmental plasticity of the male calls enables *N. triops* to communicate over a wide range of temperatures. Male call plasticity seems to represent an adaptation of the communication system to the environmental variability encountered by *N. triops* in temperate regions and likely facilitated the spread into new habitats.

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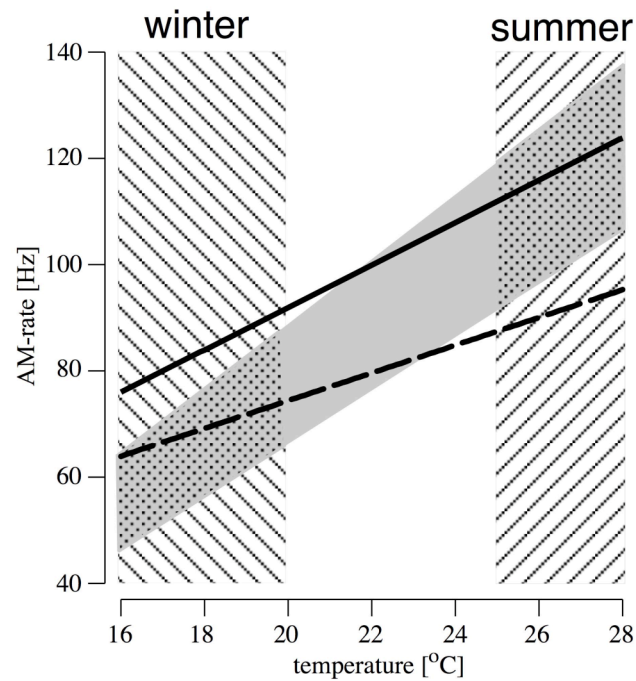


Fig. 4

Importance of call plasticity for the communication system of *N. triops*. Temperature dependency of summer call (solid line), winter call (broken line), and female AM-rate preference (light grey field). Temperature dependency of AM-rate preference based on upper cutoff and lower cutoff frequencies (at 20 and 25°C) from pooled data of summer and winter females. The AM-rate preference below 20°C and above 25°C (shaded areas) was extrapolated from our data assuming a linear relationship between temperature and preference. Hatched vertical bars indicate approximate temperature ranges at sunset between February and mid-March (left bar, winter), and between July and mid-August (right bar, summer) in Gainesville, Florida (from www.wunderground.com). Call data after Whitesell & Walker 1978

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Chapter 3

Developmental plasticity of calls in a katydid: a pre-existing male trait?

Abstract

In North America, *Neoconophalus triops* exhibits significant developmental plasticity of its mating call between summer and winter generations: at the same temperature, (1) summer calls have a higher double-pulse rate than winter calls, and (2) summer calls are structured in verses, whereas winter calls are continuous. In the tropics, where *N. triops* likely originated, males only express the summer call. We reared males from temperate and tropical populations under summer and winter conditions to investigate whether tropical populations have the capacity to express developmental plasticity in their calls. Males from tropical and temperate populations expressed developmental plasticity of double-pulse rate: double-pulse rate of winter calls was at least 20% slower than that of summer calls within each population. Only the temperate population expressed developmental plasticity of call structure: tropical populations expressed the same call structure in summer and winter calls. The difference in developmental plasticity of double-pulse rate and call structure among populations indicates independent developmental and evolutionary pathways of plasticity of these traits. Developmental plasticity of double-pulse rate likely originated in the tropics and may have predisposed *N. triops* to invade North America. The role of developmental plasticity for the diversification of the communication system and speciation is discussed.

Key words: Phenotypic plasticity, *Neoconocephalus triops*, communication, mating system

Introduction

The origin of phenotypic diversity is a fundamental question in biology. Developmental plasticity has been recognized as a potent source of phenotypic novelty (Fordyce 2006; review in West-Eberhard 2003). The evolutionary impact of developmental plasticity is especially high if it is expressed in the context of reproduction.

Communication in most animals brings the sexes together for mating and thus directly influences gene flow. Any changes of the signal, signal recognition or both can lead to rapid diversification or speciation. Developmental plasticity in communication systems therefore has the potential to be highly potent in contributing to the evolution of phenotypes and promoting speciation.

Males of the katydid *Neoconocephalus triops* use calls to attract females for mating. In Florida, *N. triops* has two reproductive generations, one in the summer (July - August, i.e. 'summer generation') and one in late winter (February - March, i.e. 'winter generation'; Whitesell & Walker 1978). Each generation is largely comprised of the offspring of the previous generation (Whitesell 1974). Calls of summer and winter males differ between generations as a result of the photoperiod experienced during juvenile development (Whitesell & Walker 1978).

First, males that reproduce in the summer (July - August, i.e. 'summer generation') produce calls with a double-pulse rate that is roughly 20% faster than those of males that reproduce in the winter (February - March, i.e. 'winter generation'; Whitesell & Walker 1978). Second, calls of summer males are structured in regularly repeated verses, whereas calls of winter males are continuous (Whitesell & Walker 1978).

Developmental plasticity of male calls plays a crucial role for communication in the different seasons by matching male calls and female preferences of the two generations (Beckers & Schul 2008). The double-pulse rate is a critical call parameter for female preference. In Florida, female preference for double-pulse rate does not differ between winter and summer females (i.e. female preference does not exhibit developmental plasticity). Nevertheless, female preference for double-pulse rate matches the rates produced by males as a result of differences between males and females in the temperature dependency of the calls and preferences: females prefer winter double-pulse rates at winter temperatures and summer double-pulse rates at summer temperatures (Beckers & Schul 2008). It remains unclear whether male call plasticity evolved in response to female preference or vice versa.

N. triops has a vast geographic distribution, ranging from Peru northward through Central America and the Caribbean to Ohio (Walker & Greenfield 1983). *N. triops* most likely originated in the tropics (see discussion) and subsequently spread from the tropics to North America (Whitesell 1974, Walker & Greenfield 1983). The expression of developmental plasticity of calls is limited to North America, where *N. triops* has two reproductive generations (Whitesell 1974). *N. triops* in the tropics reproduces all year (Greenfield 1990, Whitesell 1974) and does not encounter the short photoperiod, which

induces the winter call phenotype. Consequently, males in the tropics only express the summer call phenotype in their natural environment (Greenfield 1990, Walker & Greenfield 1983).

In the present study, we investigated whether tropical populations have the capacity to express developmental plasticity of calls if reared under summer or winter conditions. We reared *N. triops* from temperate and tropical populations in the laboratory and compared the effect of the rearing treatments on the calls among populations. We discuss the differences and similarities of developmental plasticity between tropical and temperate populations of *N. triops* in an evolutionary context.

Material and Methods

Animals and general rearing protocol

Adult *N. triops* were collected from populations in the vicinity of Gainesville (Florida), Luquillo and Naguabo (Puerto Rico), and Liberia (Costa Rica) and brought to the laboratory at the University of Missouri for egg collection. Females and males were housed on grass, which females used for oviposition. We collected eggs from the grass and stored the eggs on moist filter paper in petri dishes. Eggs were kept in the dark at 20-30°C until eclosion and sprayed daily with 0.5% Methylparaben solution to inhibit fungal growth.

The juveniles were raised on potted wheat seedlings and fed with puppy chow (Purina, MO), rolled oats, and apples. Insects were kept in incubators at 50-70% relative humidity and specific daily temperature and light cycles. A higher temperature was

maintained from one hour after lights came on until one hour before dark than during the rest of the cycle. The specific temperature and light cycles are given below.

In *N. triops* from Florida, a light/dark cycle of 15/9 h experienced during juvenile development results in summer calls (discontinuous calls with fast double-pulse rate), whereas a cycle of 11/13h results in winter calls (continuous calls with slow double-pulse rate; Whitesell & Walker 1978, Beckers & Schul 2008). Within 24h of eclosion, we randomly assigned first-instar male nymphs of each population to summer or winter rearing conditions.

Winter rearing conditions: Under winter conditions, animals were reared to adulthood at a light/dark cycle of 11/13h and high/low temperatures of 23/17°C. Two to three weeks after adult molt, we transferred these animals to a light/dark cycle of 9.5/14.5 h and temperatures of 18/12°C to secure diapause. Insects were kept under these conditions for 7-19 weeks. Diapause was terminated by increasing the day length to 13-15 h (Whitesell 1974) and high/low temperatures to 26/18°C. Termination of diapause was indicated by calling of males. We refer to animals reared under winter conditions as 'winter generation' or 'winter males' and to their calls as 'winter calls'.

Mortality during diapause differed substantially between populations. Within the first 8 weeks of diapause, 61% (14/23) of the males from Costa Rica died. 41% (22/54) of the males from Puerto Rico died within 13 weeks of diapause and 16% (5/32) of the males from Florida died within 16 weeks of diapause. We terminated diapause of the males from Costa Rica after 7-8 weeks because of their low survival rate. Note that males from Florida already express the winter call after being kept for 4 weeks in diapause and

the expression of the winter call is not influenced by duration of diapause beyond 4 weeks (Whitesell & Walker 1978).

Summer rearing conditions: Under summer conditions, animals were reared from eclosion to adulthood with a light/dark cycle of 15/9 h and high/low temperatures of 30/20°C. The males began calling 1-2 weeks after adult molt. We refer to these animals as 'summer generation' or 'summer males' and refer to their calls as 'summer calls'.

Call recordings and analysis

We recorded male calls 1-3 weeks after adult molt (summer males) or termination of diapause (winter males) in a temperature regulated anechoic chamber. Specimens were placed in screen cages (15 cm diameter) and a ¼" electret microphone (RadioShack 33-3028, frequency response 30 to 18,000 Hz) was attached to the cage. Calls were recorded with a custom-made A/D converter system (16-bit resolution, 250 kHz sampling rate). We used custom made software to measure temporal parameters of each call recording.

For each male we calculated the mean double-pulse rate of at least 100 consecutive double-pulses. To compare the degree of developmental plasticity of double-pulse rate (i.e. change of double-pulse rate between summer and winter calls) between populations, we normalized the double-pulse rate of each winter call to the grand mean of the double-pulse rate of the corresponding summer generation from the same population. All calls used in this analysis were recorded at $25.5 \pm 0.5^\circ\text{C}$.

We calculated the average verse duration of 10 consecutive verses of summer calls for each individual from recordings at $25.5 \pm 0.5^\circ\text{C}$. For winter calls, we recorded at

20 ± 1°C one calling bout of each male (i.e. from the beginning of calling until the animal ceased calling; range of bout durations: 60s to 450s) and calculated the relative proportion of the continuous part of the calling bout. We categorized parts of the calling bout as 'continuous' parts, if they were longer than 5s. All verses recorded from the summer males of the three populations were shorter than 2s.

Statistical analysis

We used the software Minitab (version 14, Minitab Inc.) for all statistical comparisons of the data. We compared double-pulse rates between generations and populations using ANOVA and post-hoc T-tests. All of these data sets fulfilled the assumptions of parametric tests (i.e. normality and homogeneity of variance). We compared verse duration of summer calls between populations using a Kruskal-Wallis test and Mann-Whitney post-hoc tests. Unless otherwise stated, data are given as mean ± standard deviation (s.d.).

Results

Developmental plasticity of double-pulse rate

Double-pulse rate of summer calls differed between populations (black bars in Fig. 1A). Males from Costa Rica produced the fastest double-pulse rates (128.1 ± 4.2 Hz, n = 27 males) and males from Florida the slowest rates (105.3 ± 5.4 Hz, n = 23). Double-pulse rates of males from Puerto Rico were in between those of the two other populations (109.8 ± 3.6 Hz, n = 28). Double-pulse rates of summer calls differed significantly among the populations (ANOVA, $F_{2,75} = 194.12$, $p < 0.0001$, all post-hoc T-tests: $p < 0.001$).

Double-pulse rate of the winter calls were about 25 Hz lower than that of the summer calls in all three populations (open bars in Fig. 1A Florida: 79.1 ± 5.3 Hz, $n = 15$; Puerto Rico: 86.4 ± 5.5 Hz, $n = 18$; Costa Rica: 102.1 ± 5.9 Hz, $n = 6$). These differences were highly significant (all three populations: T-test, $p < 0.0001$).

Normalized double-pulse rates (see above) of winter calls were between 24.87% (Florida) and 20.28% (Costa Rica) lower than that of the summer calls at the same temperature (Fig. 1B). Thus, tropical and temperate populations both have the capacity for developmental plasticity of double-pulse rate. The difference in the normalized double-pulse rates of winter calls between populations was marginally significant (ANOVA, $F_{2,36} = 2.78$, $p = 0.075$).

Developmental plasticity of call structure

Summer males produced calls that were structured in verses. Summer males from Florida and Puerto Rico produced calls with similar verse durations (Florida: 1004.4 ± 110.2 ms, Puerto Rico: 937.3 ± 89.2 ms, each $n = 10$), whereas males from Costa Rica produced significantly shorter verses than the other two populations (610.5 ± 72.1 ms; Kruskal-Wallis test: $H_{10,10,10} = 20.60$, $p < 0.0001$, post-hoc Mann-Whitney test: both CR vs. FL and CR vs. PR: $U_{10,10} = 155.0$, $p < 0.0002$; FL vs. PR: $U_{10,10} = 127.0$, $P = 0.104$). No summer male produced a verse that was longer than 2 s in duration.

Call structure of the winter calls differed between populations. Winter males from Florida ($n = 6$) produced continuous calls with only a brief sequence of verses at the beginning and the end of the calling bout (Fig. 2, top panel); on average, 83.1% of each calling bout consisted of continuous rather than versed calls (Fig. 3). In contrast, winter

calls of males from Puerto Rico and Costa Rica were structured in verses throughout the duration of the calling bout (Fig. 2, 3); we never observed an uninterrupted call in these populations (n = 6 males for both populations). Thus, the developmental plasticity of call structure was limited to the population from Florida.

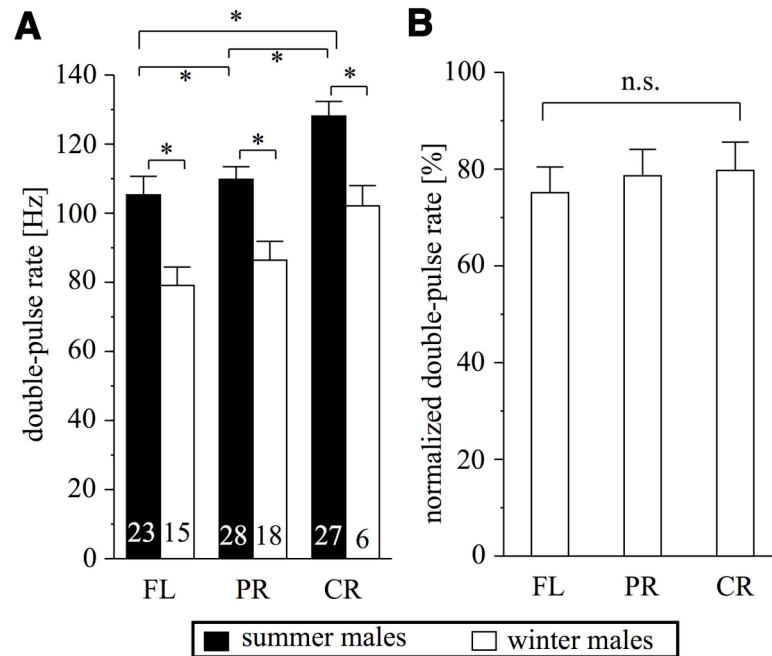


Fig. 1

(A) Double-pulse rates (mean + s.d.) of males raised under summer (black bars) and winter conditions (open bars) from Florida (FL), Puerto Rico (PR), and Costa Rica (CR). The double-pulse rates differed significantly among summer calls of the three populations (ANOVA: $F_{2,75} = 194.12$, $p < 0.0001$); calls of the CR population were faster than calls of the PR and FL population (post-hoc T-tests: FL vs. CR $t_{48} = -16.80$, $p < 0.001$; PR vs. CR $t_{53} = -17.16$, $p < 0.001$) and calls of the PR population were faster than calls of the FL population (post-hoc T-test: FL vs. PR: $t_{49} = -3.61$, $p < 0.001$). In all three populations, calls of the winter generation were significantly slower than calls of the summer generation (T-test: FL $t_{36} = 14.75$, $p < 0.001$; PR $t_{44} = 17.52$, $p < 0.001$; CR $t_{31} = 12.68$, $p < 0.001$). (B) Normalized double-pulse rates (mean + s.d.) of males raised under winter conditions as percentage of the mean double-pulse rate of the summer calls

of each population. The normalized double-pulse rates did not differ significantly among the three populations (ANOVA, $F_{2,36} = 2.78$, $p < 0.075$). Call recordings were conducted at an ambient temperature of $25.5 \pm 0.5^\circ\text{C}$.

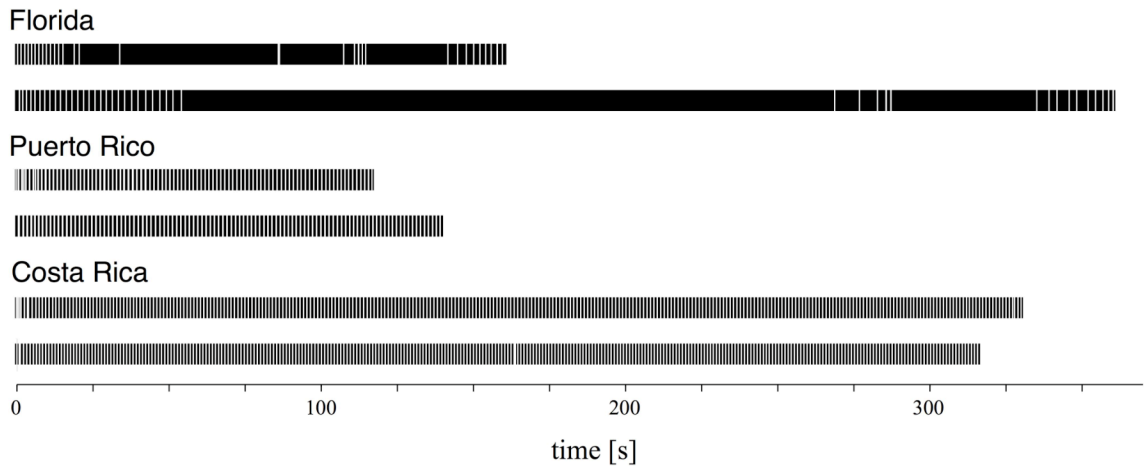


Fig. 2

Call structure of males from Florida, Puerto Rico, and Costa Rica that were raised under winter conditions. The timing of representative calling bouts (= from the beginning of calling until male ceased calling) of two males is shown for each population. Black bars indicate time when sound was produced; white lines represent the occurrence of silent gaps. Males were recorded at $20 \pm 1^\circ\text{C}$ ambient temperature.

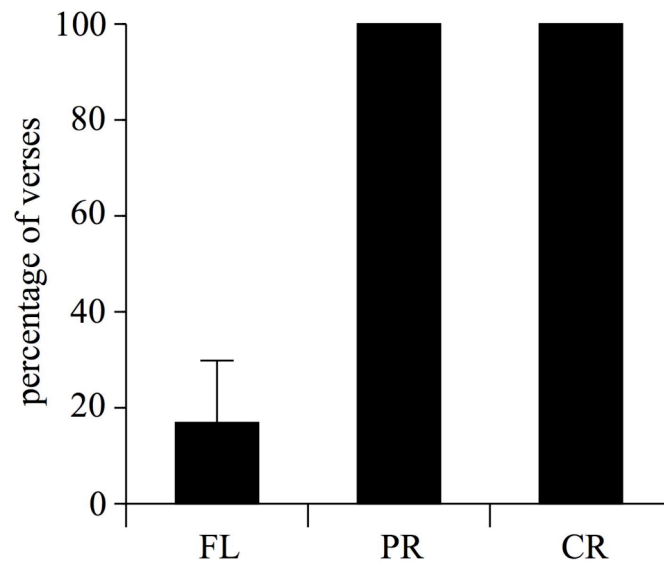


Fig. 3

Percentage (mean + s.d.) of calls structured in verses (= discontinuous calls) of males that were reared under winter conditions (Florida = FL, n = 6; Puerto Rico = PR, n = 6; Costa Rica = CR, n = 2). Uninterrupted calling of 5s and longer was classified as continuous calling, shorter call pieces as calling with verses. Males were recorded at $20 \pm 1^\circ\text{C}$ ambient temperature.

Discussion

Our experiments revealed that the developmental plasticity for double-pulse rate was shared among tropical and temperate populations: the double-pulse rate of winter males was at least 20% slower than that of summer males in all three populations (Fig. 1). In contrast, developmental plasticity of the call structure was limited to the population from Florida (Fig. 3). These results indicate that developmental plasticity for double-pulse rate and for call structure are separate traits with separate developmental pathways and evolutionary trajectories.

The life history of *N. triops* with direct egg development and adult diapause resembles that of tropical species, whereas temperate *Neoconocephalus* species diapause as eggs (Greenfield 1990). This pattern suggests that *N. triops* is a 'tropical' species that moved secondarily to North America (Whitesell 1974, Greenfield 1990). A molecular analysis of the population genetics of *N. triops* supports this interpretation (R. Snyder, pers. comm.), which we adopt in our arguments regarding the evolution of the communication system in *N. triops*.

Previous studies have shown that female *N. triops* from Florida are not selective for verse structure: both continuous and versed calls were highly attractive to both summer and winter females (Beckers & Schul 2008). Therefore, developmental plasticity of verse structure, which was only present in the Florida population, is likely to have little, if any, importance for intraspecific communication in this population (Beckers & Schul 2008).

Females from Costa Rica, on the other hand, require male calls with verse structure: continuous calls, as produced by winter males from Florida, are not attractive

(OMB, unpublished results). Assuming a tropical origin of *N. triops*, male calls and female preference of the Costa Rica population most likely resemble the ancestral state in *N. triops*. After *N. triops* spread to North America, developmental plasticity of call structure could only evolve after (or concomitantly with) the loss of female preference for the reversed calls. Whether the loss of preference and/or the gain of call plasticity were adaptive or due to non-selective processes remains unclear. One possibility is that the changes occurred as a by-product of physiological adaptations to the seasonal climate encountered in North America. Mortality rates during diapause were much higher for the tropical populations (see methods), indicating major adaptations of the Florida population to these conditions.

The two tropical populations tested here exhibited the same potential for developmental plasticity of double-pulse rate as the Florida population (Fig. 1), suggesting that this plasticity was already present when *N. triops* spread from the tropics to North America. The alternative scenario that plasticity of double-pulse rate evolved in North America and spread through gene flow into tropical populations appears unlikely. Minimal gene flow between the North American and tropical populations is suggested by 1) the differences in absolute double-pulse rate among the three populations (Fig. 1a), and 2) the existence of developmental plasticity of call structure in the North American population but not in the tropical populations (Fig. 2, 3).

Developmental plasticity can be adaptive for living in recurrently variable environments (Yeh & Price 2004, Trussel & Smith 2000, Laurila et. al 2002) and may facilitate the spread into new environments (Yeh & Price 2004, Ehrlich 1989, Holway &

Suarez 1999). In Florida, *N. triops* encounters a distinctly larger temperature range across seasons than in the tropics (Beckers & Schul 2008). Both double-pulse rate of the male calls and female preference for double-pulse rate are temperature dependent. However, the temperature dependency of female preferences is significantly steeper than that of male double-pulse rate, resulting in an overlap between call and preference in only a relatively narrow temperature range (Beckers & Schul 2008). Because of developmental plasticity of double-pulse rate, communication works over the wide temperature range across seasons: winter calls are attractive to females at low temperatures and summer calls at high temperatures (Beckers & Schul 2008). In this sense, developmental plasticity of double-pulse rate in tropical populations may be interpreted as enabling *N. triops* to invade temperate regions. This view is supported by the fact that *N. triops* is the only tropical species with a range extending into temperate zones.

Alternatively, one might argue that the temperature dependency of male summer calls and female preference in the ancestral tropical population were probably matched in steepness over a wide range of temperatures. The pre-existing developmental plasticity of double-pulse rate would complicate the invasion of North America because the double-pulse rate of (newly-expressed) winter calls would be below the preferred double-pulse rate of females. The developmental plasticity of male calls would have exerted selection pressure on females to increase the steepness of the temperature dependency of their preferences. According to this scenario, females of the North American population adapted to the plasticity of male double-pulse rate. Comparative studies of female preference among temperate and tropical populations may elucidate whether the pre-

existing plasticity in male calls facilitated or hindered the spread of *N. triops* into North America.

Following the invasion of a new habitat, developmental plasticity may no longer be expressed due to consistent environmental induction of only one phenotype (Shapiro 1976, West-Eberhard 1989). Developmental plasticity could then be lost due to either selection against plasticity (Pfennig & Murphy 2000, West-Eberhard 2003) or genetic drift (West-Eberhard 1989). In the northernmost part of its distribution range (e.g. Ohio), *N. triops* reproduces only once per year and males express only the winter call phenotype (Whitesell 1974, Walker 2001), most likely because they never experience the environmental conditions inducing the summer call. Genetic fixation (= genetic assimilation; Waddington 1961, Pigliucci et al. 2006) of the winter call phenotype could lead to reproductive isolation between northern and southern populations of *N. triops*. Thus, developmental plasticity of male calls in *N. triops* has the potential to lead ultimately to speciation.

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Chapter 4

Male call plasticity caused the evolution of female preference in the communication system of *Neoconocephalus triops* (Orthoptera: Tettigoniidae)

Abstract

In North America, *Neoconocephalus triops* expresses substantial developmental plasticity in the double-pulse rate of mating calls between summer and winter generations. Here, temperature dependency of female preference for double-pulse rate is steeper than that of either male call, matching preference to the summer call at high temperatures and the winter call at low temperatures. Tropical populations only express the summer call in their environment. We compared steepness of temperature dependency of female preference between temperate and tropical populations of *N. triops* to test if the steep temperature dependency of preference in North America represents an adaptation to call plasticity or a shared trait among all populations. The change of preference with temperature was significantly steeper in the population from Florida compared to tropical populations and other *Neoconocephalus* species. Temperature dependency of *N. triops* calls, however, was similar to that of other *Neoconocephalus* species. These results indicate that female preference evolved in response to the expression of the additional winter call in the Florida population. This adaptation of preference suggests that call plasticity complicated the spread of *N. triops* from the tropics to North America.

Introduction

Acoustic communication systems of insects and anurans exhibit an impressive diversity of mating signals (Gerhardt & Huber 2002). This is especially remarkable, considering that male signals and female preference have to co-evolve for the communication system to remain functional. This diversity is generally explained by sexual selection, with female choice driving the evolution of male signals (Endler & Basolo 1998, West-Eberhard 1983, Andersson 1994, Rodriguez et al. 2006).

The katydid *Neoconocephalus triops* is a tropical species with a range extending into North America (Whitesell 1974; Greenfield 1990). In North America, males exhibit substantial developmental plasticity of mating calls between summer and winter generations. Foremost, amplitude modulation (AM-) rate of summer calls is more than 20% faster than that of winter calls at the same ambient temperature (Whitesell & Walker 1978). Tropical populations of *N. triops* express only the fast summer call phenotype in their natural environment (Greenfield 1990, Walker & Greenfield 1983).

Female *N. triops* have a stabilizing preference for AM-rate (Beckers & Schul 2008, Whitesell & Walker 1978). Female preference does not exhibit developmental plasticity that parallels that of male calls (Beckers & Schul 2008). However, preference for AM-rate changes significantly more with temperature than the AM-rate of male calls. This steeper temperature dependency of the female preference results in an overlap of the female preference with the summer call at high (= summer) temperatures, and the winter call at low (= winter) temperatures, facilitating communication in both seasons (Beckers & Schul 2008).

Males from tropical populations have the capacity to express both call phenotypes, even though environmental conditions in the tropics do not induce the expression of the winter call phenotype (Beckers & Schul subm.). Thus, developmental plasticity of male calls was likely already in place when *N. triops* expanded its range to North America. However, it is not clear whether the steep temperature dependency of female preference is shared among all *N. triops*, or is limited to populations that express call plasticity. There are two possible evolutionary hypotheses: (1) The steepness of the temperature dependency of female preference increased in North America to compensate for the seasonal differences of male calls. This scenario predicts a steeper temperature dependency of temperate *N. triops* compared to tropical populations of *N. triops* as well as other *Neoconocephalus* species. (2) Alternatively, the steep temperature response of preference may be a shared trait of all female *N. triops* and therefore be a predisposition for living in the seasonal climate of North America as previously suggested (Beckers & Schul 2008). This hypothesis predicts no difference in female preference between temperate and tropical populations of *N. triops*.

Here we test female preferences of a temperate and two tropical populations of *N. triops*, as well as those of three additional *Neoconocephalus* species, to distinguish between these two hypotheses. The temperature dependency of female preference was steepest in temperate *N. triops*, while the temperature dependency of tropical *N. triops* was similar to that of the other three species. This indicates that female preference of Florida *N. triops* evolved in response to the male call.

Material & Methods

Animals

Choice of populations/species. We tested female preference of three *N. triops* populations: Florida, a temperate population which expresses call plasticity, and Puerto Rico and Costa Rica, two tropical populations which do not express male call plasticity under natural conditions. We also tested females of three additional species (*N. affinis*, *N. bivocatus*, *N. robustus*), chosen to represent a wide range of AM-rates of the male calls (approximately 200 Hz in *N. robustus*, 80-90 Hz in *N. bivocatus*, and 11-13 Hz in *N. affinis*; Greenfield 1990). Females of *N. bivocatus* and *N. affinis* use the AM-rate to identify conspecific calls (Deily & Schul 2004; Bush et al. in prep.), as does *N. triops* (Beckers & Schul 2008). Female *N. robustus* use the duration of the silent interval between pulses for call recognition, which likely represents the ancestral state in this genus (Deily & Schul 2004).

N. triops: We collected adult male and female *N. triops* from populations in the vicinity of Gainesville (Florida), Liberia (Costa Rica), Luquillo and Naguabo (Puerto Rico), and brought the animals to the laboratory at the University of Missouri for egg collection. We collected eggs from these animals and raised the offspring using the protocol described in Beckers and Schul (2008).

Offspring of the Puerto Rico and Costa Rica populations were raised under 'summer conditions' (Beckers & Schul 2008); offspring of the Florida population were raised both under summer (n = 11) and winter conditions (n = 9). Female preferences did not differ between animals raised under different conditions (Beckers and Schul 2008), so we pooled the phonotaxis data of both groups.

N. affinis: Adult *N. affinis* were collected in Puerto Rico in the vicinity of Naguabo and Luquillo. Offspring of these animals were raised using the same protocol as the *N. triops* from Puerto Rico and Costa Rica.

N. bivocatus and *N. robustus*: Females of these two species were collected as last instar nymphs in Boone County, Missouri (USA). The insects were kept at a light/dark cycle of 14/10 h at 28/20°C until maturity.

We started testing of females of all species two to three weeks after adult molt. Females were tested for up to six weeks, during which we detected no changes in their selectivity.

Call recordings

We recorded male calls of *N. affinis* (n = 21) and of *N. triops* from Florida (n = 13), Puerto Rico (n = 13), and Costa Rica (n = 16) between 22 and 28°C. Calls were recorded with a custom-made A/D converter system (16-bit resolution, 250 kHz sampling rate) and ¼" electret microphones (RadioShack 33-3028, frequency response 30 to 18,000 Hz). For each male we calculated the mean AM-rate of at least 100 consecutive double-pulses. We calculated linear regression lines of AM-rates for each population. Regression coefficients were normalized to the value at 25°C for each population to determine the relative change of AM-rate per degree Celsius. Previously reported call data for *N. bivocatus*, *N. robustus* and *N. triops* (Florida) was converted to be comparable with our call data (Tab. 1).

Female phonotaxis tests

We tested female phonotaxis using a walking compensator (Kramer-Kugel, Weber et al. 1981) in a temperature-regulated anechoic chamber at ambient temperatures between 20°C and 29°C. In short, females were placed on top of a sphere, free to walk but kept in place by compensatory sphere rotations, while acoustic signals were presented from loudspeakers located in the animal's horizontal plane. The intended direction and speed of the animal were read from the control circuitry. All experiments were performed in the dark except for the infrared light used to monitor the insects' position. (For details see Schul 1998; Weber et al. 1981).

Experimental protocol: Each test and control stimulus was presented for 60-90 s from each of two different loudspeaker positions. The first stimulus of each test series was the control stimulus, followed by two to three test stimuli, another control, etc. A period of 60 s of silence was given between stimuli. The experimental series lasted between 30 and 60 min and consisted of up to six test stimuli (plus up to four controls). We randomly varied the sequence of stimuli within a series among individual females. (For a detailed description of the experimental protocol see Schul 1998, Bush et al. 2002).

Stimulation: We generated synthetic signals using a custom-developed DA-converter/amplifier system with 250 kHz sampling rate and 16-bit resolution. Stimuli were delivered using one of two loudspeakers (Motorola KSN1218C) mounted at a distance of 150 cm in the horizontal plane of the animal and separated by an angle of 105°. Signal amplitude was set to 80 ± 2 dB peak SPL (re. 20×10^{-5} Pa) using a Bruel and

Kjaer sound level meter (B&K 2231) and a ¼" condenser microphone (G.R.A.S 40BF) which was positioned 1 cm above the sphere.

For stimulus generation, we used pure tones as a carrier signal to which we subsequently applied amplitude modulations: *N. robustus*: 7 kHz, *N. bivocatus*: 10 kHz (Schul & Patterson 2003), *N. triops*: 11 kHz (Beckers & Schul 2008), *N. affinis*: 12.5 kHz & 25 kHz at -6 dB relative to 12.5 kHz (Bush et al. in prep.). All test stimuli were continuous, except those for the experiments with *N. triops* from Costa Rica because females were only attracted to interrupted call models (unpublished data). Calls of *N. triops*, *N. bivocatus*, and *N. affinis* consist of double-pulses (Walker et al. 1974, Whitesell & Walker 1978, Walker & Greenfield 1983). Females of these three species respond equally strong to stimuli with double-pulses merged to long pulses (Deily & Schul 2004, Beckers & Schul 2008, Bush et al. in prep.). Stimuli for experiments with these species consisted of pulses equivalent to a 'merged double pulse' of the male call. We refer to the double-pulse rate of male calls and the equivalent rate of the long pulses in our stimuli as AM-rate. Male *N. robustus* produce calls with a single-pulse structure (Walker et al. 1974). Here stimuli mimicked this pattern and, AM-rate refers to the pulse rate in male calls and stimuli used. For each species, we adjusted pulse duration and interval duration to generate stimuli that varied in AM-rate.

The control stimulus for each tested temperature had the AM-rate of the conspecific male call at this temperature. Control stimuli for all species except *N. triops* were continuous. For experiments with *N. triops* from Florida and Puerto Rico, control stimuli had verse/interval durations of 950/50 ms (Beckers & Schul 2008) and those for

experiments with *N. triops* from Costa Rica had durations of 650/50 ms (Beckers & Schul submitted).

Data analysis: We quantified female response to stimuli that varied in AM-rate by calculating a phonotaxis score (Schul 1998). The phonotaxis score included three measures indicating the relative strength of phonotaxis: (1) the walking speed relative to the walking speed during the control stimulus, describing the elicited locomotion activity; (2) the vector length, describing the accuracy of orientation; and (3) the angular orientation relative to that during the control stimulus. The score can range from approximately +1 to -1, representing perfect positive or negative phonotaxis, respectively (for details see Schul 1998). For each female, we plotted phonotaxis scores as a function of AM-rate for each temperature. These response functions were smoothed by calculating a gliding average through three values and then normalized (Beckers & Schul 2008).

Because of the different call recognition mechanisms, the shape of female response functions (= preference) differed between species. Preference functions of species using AM-rate for call recognition peaked at certain AM-rates and decreased towards both lower and higher AM-rates, i.e. had band-pass characteristics (Fig. 1). *N. robustus* uses the interval duration for call recognition (Deily & Schul 2004) and the preference function decreased only towards lower AM-rates (= longer intervals) but not towards higher AM-rates (= shorter intervals; Fig. 1), i.e. had high-pass characteristics. We fitted parabolic functions ($y = c - a*(x-b)^2$) on the phonotaxis scores of each female with 'closed' preferences and sigmoidal functions ($y = 1/1+e^{(-x+a)*b}$) on the scores of female *N. robustus* by minimizing the sum of squared errors (Beckers & Schul 2008).

We used for all four species the AM-rate at which the function fell below 70% towards lower AM-rates (= lower cutoff rate) as an estimate for the tuning of female preference. We determined the lower cutoff rate for each female and used the change of this AM-rate with temperature to estimate the temperature dependency of female preference. This approach allowed us to compare temperature dependency of female preference between species.

Statistics: We obtained preference data for each *N. triops* only at one temperature (20°C or 25°C), i.e. the tuning data for this species consisted of independent data points. To compare temperature dependency of female preference between populations of *N. triops*, we normalized the individual lower cutoff rates to the population mean at 25°C and calculated linear regression lines for each population. Regression coefficients indicated the relative change of female preference [%/°C] and were compared among populations using an Analysis of Covariance and post-hoc pair-wise Tukey tests (Zar 1981).

For *N. affinis*, *N. bivocatus*, and *N. robustus*, we determined preference functions at two to three temperatures (22, 25, 29°C) for each female.

We determined the temperature dependency of by normalizing the lower cutoff rates to the mean value of each species at 25°C and calculating a linear regression line for *each* female. We calculated for each species the mean value of these individual regression coefficients.

To compare the temperature dependency of *N. triops* to the other species (Fig. 3), we calculated for each *N. triops* female tested at 20°C the difference between its normalized lower cutoff rate and the mean normalized rate at 25°C. From this difference,

we calculated the relative change with temperature ($\%/^{\circ}\text{C}$). Population/species means were compared using a Kruskal-Wallis test and post-hoc Mann-Whitney tests (Zar 1981).

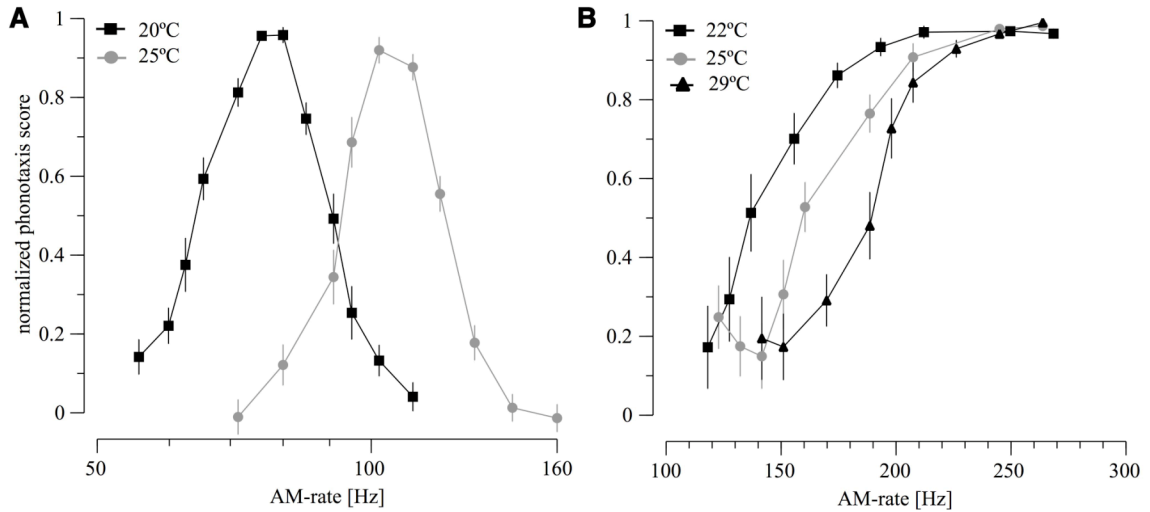


Fig. 1

Representative examples of female preference functions of species using (a) AM-rate or (b) interval duration for call recognition. (a) The preference functions of *N. triops*, *N. affinis*, *N. bivocatus* have 'band-pass' characteristics, i.e. phonotaxis scores are high within a range of AM-rates but decrease towards faster and slower rates (function of *N. triops* from Puerto Rico is shown). (b) The preference function of *N. robustus* has 'high-pass' characteristics, i.e. phonotaxis scores decrease only towards slow AM-rates.

Results

Comparison of N. triops populations

With increasing temperatures, preference functions of female *N. triops*, as well as of the other three species, shifted towards faster AM-rates (Fig. 1). The relative change of female preference differed among the three populations of *N. triops* (Fig. 2). The linear regression of the temperature dependency was the steepest for the Florida population (5.82%/°C), and were less steep for the two tropical populations (Costa Rica: 4.10%/°C, Puerto Rico: 4.91%/°C). The regression coefficients differed significantly among the three populations (ANCOVA: $F_{2,64} = 4.697$, $0.02 < p < 0.05$) with the Florida population being significantly steeper than the Costa Rica population (post-hoc Tukey test: $k = 3$, $DF = 64$, $q = 3.938$, $0.01 < p < 0.025$). The regression coefficient of the Puerto Rico population was not significantly different from either the Florida or the Costa Rica population (post-hoc Tukey tests: Puerto Rico vs. Florida: $k = 3$, $DF = 64$, $q = 2.1544$, $0.2 < p < 0.5$; Puerto Rico vs. Costa Rica: $k = 3$, $DF = 64$, $q = 1.8743$, $0.2 < p < 0.5$).

Comparison to other species

The previous result was in agreement with the hypothesis that female preferences of temperate *N. triops* changed in response to male call plasticity that was expressed when the species extended its range to Florida. If this is true, then the temperature dependency of female preferences of the Florida population should also differ from other *Neoconocephalus* species.

The steepness of temperature dependency of female preference of *N. bivocatus* (3.88 ± 1.26 %/°C, $n = 6$), and *N. robustus* (4.10 ± 2.04 %/°C, $n = 9$) was similar to that of

the Costa Rica population of *N. triops*, whereas the steepness of *N. affinis* (3.10 ± 0.98 %/°C, n = 10) was somewhat lower (Fig. 3).

Statistical analysis of all six data sets revealed that the steepness of the *N. triops* from Florida was significantly higher than that of all other populations of *N. triops* and the other three species (Kruskal-Wallis test: DF = 5, H = 34.45, p = 0.0001, all post-hoc Mann-Whitney tests between Florida *N. triops* and the other species and populations: p < 0.02, see Fig. 3). Temperature dependency of female preference did not differ significantly among the tropical *N. triops* populations, *N. bivocatus*, and *N. robustus* (all post-hoc Mann-Whitney tests: p > 0.1). The temperature dependency of *N. affinis* was significantly less steep than that of all three *N. triops* populations (all post-hoc Mann-Whitney tests: p < 0.02).

The difference in steepness between *N. triops* from Florida and other *Neoconocephalus* species also supported the scenario that female preference adapted to the male call plasticity expressed in temperate habitats.

Temperature dependency of male calls

Temperature dependency of AM-rate of male calls varied for different measurements and populations of *N. triops* between 1.88%/°C and 3.60%/°C (table 1). Differences between studies were likely due to relatively small sample sizes (N = 13-16, Tab. 1). The temperature dependency of *N. triops* calls were in the same range as those of other *Neoconocephalus* species (2.27 %/°C to 3.61 %/°C, Tab. 1).

Thus, the steepness of temperature dependency of male calls was in a similar range as that of female preferences of *N. triops* from Costa Rica and the three other

Neoconocephalus species. However, temperature dependency of female preference of the *N. triops* Florida population was substantially steeper than that of male calls.

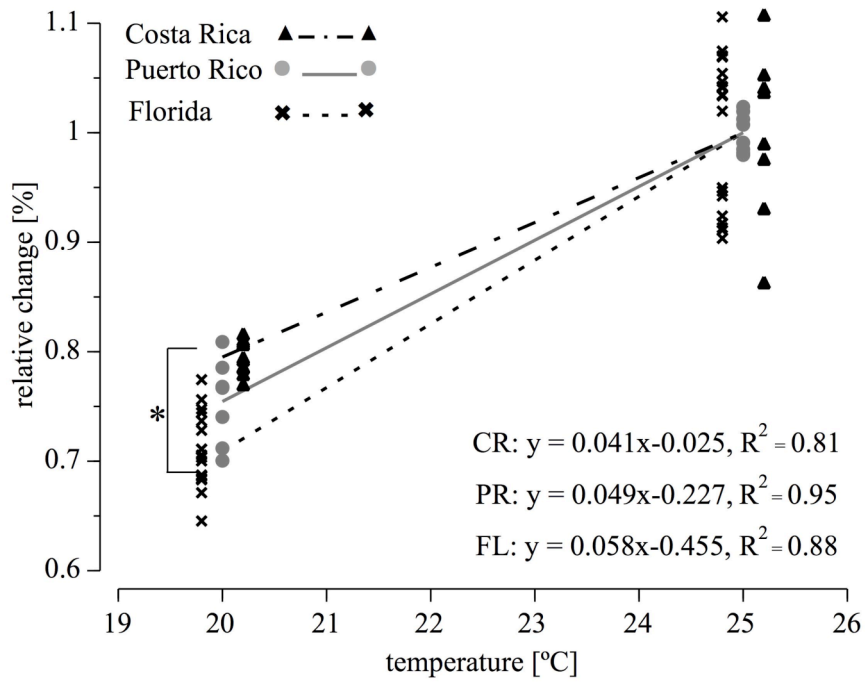


Fig. 2

Relative temperature dependency of female preference for populations of *N. triops*.

Symbols indicate relative cutoff rates of individual females tested. Regression lines were calculated based on these data. Formulas of regression lines are given at bottom right corner of figure (CR = Costa Rica, PR = Puerto Rico, FL = Florida). Change of female preference with temperature of the population from Florida was significantly steeper (indicated by asterisk) than that of the population from Costa Rica (ANCOVA: $F_{2,64} = 4.697$, $0.02 < p < 0.05$; post-hoc Tukey test: Florida vs. Costa Rica: $q = 3.938$, $0.01 < p < 0.025$).

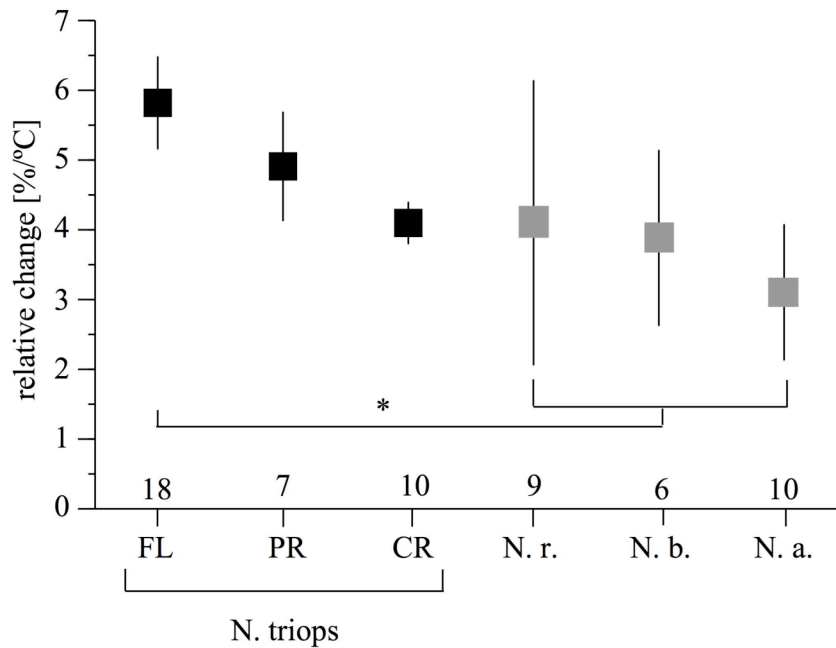


Fig. 3

Average relative change (\pm s.d.) of female preference per degree Celsius of *N. triops* from Florida (FL), Puerto Rico (PR), Costa Rica (CR), *N. robustus* (N. r.), *N. bivocatus* (N. b.), and *N. affinis* (N. a.). Asterisk indicates significant difference in relative change of female preference between *N. triops* from Florida and other species of *Neoconocephalus* (Kruskal-Wallis test: DF = 5, H = 34.45, p = 0.0001; post-hoc Mann-Whitney tests: FL vs. CR: W = 350.0, p < 0.001; FL vs. N. r.: W = 308.0, p = 0.0043; FL vs. N. b.: W = 271.0, p = 0.024; FL vs. N. a.: W = 350.0, p < 0.001). N for each population/species given at bottom of figure.

species	Change pulse rate of call relative to 25°C [%/°C]	Linear regression of male calls	Source for call data
<i>N. triops</i> (Florida)	S: 1.88	$y = 2.07x + 58.22$ (N = 13)	This study
	S: 3.60	S: $y = 3.99x + 12.13$ (N = 15)	Walker & Whitesell (1978)
	W: 2.98	W: $y = 2.61x + 22.19$ (N = 16)	
<i>N. triops</i> (Puerto Rico)	3.61	$y = 3.75x + 10.19$ (N = 13)	This study
<i>N. triops</i> (Costa Rica)	1.88	$y = 2.39x + 67.45$ (N = 16)	This study
<i>N. robustus</i>	2.27	$y = 4.7x + 89.9$	Büttner (2002)
<i>N. bivocatus</i>	4.32	$y = 3.8x - 6.9$	Büttner (2002)
<i>N. affinis</i>	3.61	$y = 0.45x + 1.19$	This study

Tab. 1

Temperature dependency of male calls of *Neoconocephalus* species. Change of male calls was calculated relative to AM-rate at 25°C. Formulas for temperature dependency of AM-rates and literature sources are indicated.

Discussion

We compared the temperature dependency of female preference of Florida *N. triops* to tropical populations and other *Neoconocephalus* species. Female preference of *N. triops* from Florida changed significantly more with temperature than the preference of tropical populations or all the other species tested (Fig. 2, 3). Temperature dependency of calls of all *N. triops* populations was similar to that of other *Neoconocephalus* species (Tab. 1).

Calls of most *Neoconocephalus* species are exceptionally fast with pulse (=wing-stroke) rates of 150-250 Hz (including *N. triops*; Greenfield 1990). In these species, the heat produced during calling elevates the thorax temperature well above ambient temperature (Heath & Josephson 1970, Josephson 1984), resulting into comparatively little change of AM-rate with changing ambient temperature (Walker 1975).

Even though silent females do not produce heat, temperature dependency of female preference appears to be reasonably well matched to that of male calls in *N. triops* from Costa Rica and the other three species tested here. This match indicates the adjustment of female preference to the inevitable temperature dependency of male calls (Helvesen & Helvesen 1994) and facilitates communication over a broader temperature range.

In contrast, female preference changed substantially more with temperature than male calls for the Florida population of *N. triops*. This steep temperature dependency, however, is necessary to cause the match between female preference and the summer call at high temperatures and the winter calls at low temperatures (Beckers & Schul 2008).

N. triops is a tropical species that expanded its distribution range into North America (Whitesell 1974). In the tropics, *N. triops* expresses only the summer call (Greenfield 1990, Walker & Greenfield 1983), even though males likely had the capacity for developmental plasticity of double-pulse rate (Beckers & Schul submitted). Temperature dependency of female preference of tropical *N. triops* was probably adjusted to that of the summer call, as suggested by our results. The spread to North America exposed *N. triops* to temperate environmental conditions that induce the additional expression of the winter call in the winter generation. The winter call, however, has a substantially slower double-pulse rate than the summer call (~20% slower than summer call; Whitesell & Walker 1978, Beckers & Schul submitted), resulting into a mismatch between female preference and the winter call at low temperatures (Fig. 4a). This mismatch leads to reduced female attraction to winter calls and thus a slow phonotactic approach towards winter males. Developmental plasticity of male calls, however, was retained in the temperate population and did not change in its magnitude between summer and winter calls (capacity of ~20% change of double-pulse rate in all temperate and tropical populations tested; Beckers & Schul submitted). Instead, temperature dependency of female preference became steeper, matching each call phenotype within a narrow temperature range in each season, and thus promoting communication also in the winter (Fig. 4b).

Thus, female preferences of *N. triops* have seemingly adapted in response to a property of the male call, i.e. the developmental plasticity of double-pulse rates. This conclusion is counterintuitive in the context of sexual selection theory, which predicts

male signals to follow female preferences rather than vice versa (e.g. Andersson 1994, Endler & Basolo 1998, Rodriguez et al. 2006).

Following this mindset, we interpreted our initial finding that each call phenotype matches female preference in the Florida population in the appropriate season (Beckers & Schul 2008), as an adaptation of male calls to female preferences. However, considering our later findings that developmental plasticity is present in tropical population (although not expressed) (Beckers & Schul submitted) and that female preferences of the Florida population differ from those of tropical populations (this study) lead to the opposite (and unexpected) conclusion that females preferences adapted to male calls. This highlights the need to challenge conventional wisdom when formulating testable hypotheses to explain evolutionary processes.

The conditions in the winter generation are substantially different from those of the summer generation, possibly exerting strong selection on females for efficient and fast phonotaxis. Early at night, winter males frequently change calling sites, likely to avoid parasitism by acoustically orienting flies (Burk 1982, Whitesell 1969). This leaves females only short time windows to approach a specific male. Later at night, temperatures close to the ground drop faster than at higher elevations, so that calling activity is then limited to males in elevated calling sites (Whitesell 1969, personal observation). Males in such positions often have wide broadcasting ranges (Arak & Eiriksson 1992, Marten & Marler 1977) and therefore may attract females from a wide area. Winter males likely mate only once per night (Whitesell 1969, Gwynne 1977). These conditions may lead to competition of females to be the first to reach one of these

few calling males in elevated sites. Ambient temperature limits the duration of the chorus in the winter generation (< 4 h, personal observation) so that it may not be possible for the losers of this competition to approach another male. Thus, 'scramble competition' (Andersson & Iwasa 1996) among females may have caused the adaptation of female preferences to the male call plasticity in temperate *N. triops*.

The reason why the winter calls did not adjust to female preference remains unclear. The decrease of the double-pulse rate of the winter call could be the result of a pleiotropic effect that also influences the expression of traits that increases fitness when expressed in the winter. Selective pressure may be stronger on these traits than that on the slower double-pulse rate of the winter call, preventing the independent evolutionary change of the rate (Newman 1994, Lande 1982).

Developmental plasticity can play an important role in trait evolution: it can either generate phenotypic variation for selection to act on, or plasticity itself can respond to selection (West-Eberhard 2003, Agrawal 2001, Pigliucci 2005). Developmental plasticity in the communication system of *N. triops* led to evolutionary change in a different way. Expression of plasticity in one trait (male calls) exerted selective pressure on a related trait (female preference), causing it to evolve (increase of steepness of temperature dependency).

Developmental plasticity can also play an important role in ecology: the ability to express more than one phenotype may be of ecological significance if it is crucial for survival in recurrently variable environments (Yeh & Price 2004, Trussel & Smith 2000,

Laurila et. al 2002), or facilitates the expansion into new environments (Yeh & Price 2004, Ehrlich 1989, Holway & Suarez 1999). In *N. triops*, however, developmental plasticity of male calls complicated communication in the different seasons of North America and likely hindered the extension of the distribution range to the North.

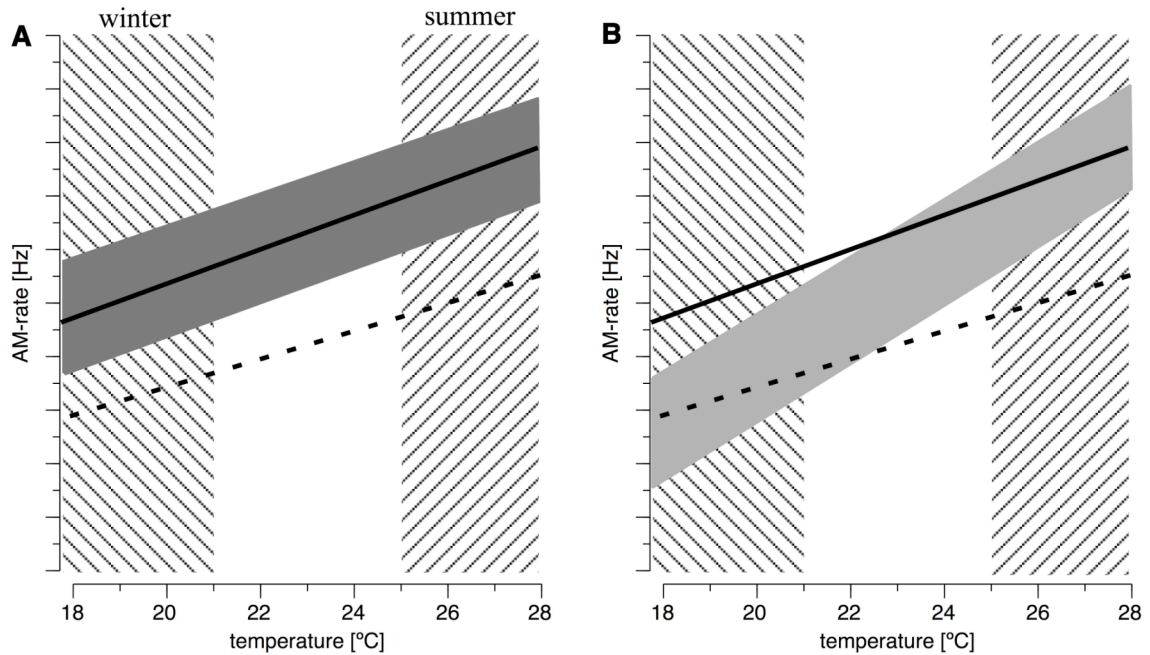


Fig. 4

Hypothetical model of the evolution of female preference in the population of *N. triops* from Florida. The summer call is indicated by the solid line, the winter call by the dashed line, and hatched vertical bars indicate approximate temperature ranges after sunset for the summer and winter generation. (A) Dark grey area indicates likely ancestral temperature dependency of female preference. (B) Light grey area indicates temperature dependency of female preference after adaptation to male call plasticity.

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Chapter 5

GENERAL DISCUSSION

Summary of results

Developmental plasticity can be a potent initiator of phenotypic change and speciation. The katydid *Neoconocephalus triops* expresses substantial developmental plasticity in its reproductive communication system. In North America, male call phenotypes differ substantially between the summer and the winter generation in double-pulse rate and call structure. In the tropics, where *N. triops* originated, male calls do not exhibit call plasticity in this environment.

Rearing experiments showed that males of both temperate and tropical populations had the same capacity for developmental plasticity of double-pulse rate. However, only males from temperate populations also displayed the capacity for plasticity of call structure.

Females of temperate and tropical populations had strong preferences for double-pulse rate and used this parameter for call recognition. Females from Florida and Puerto Rico exhibited strong responses to both versed and continuous calls. However, females from Costa Rica were only attracted to versed calls.

Temperature dependency of female preference for double-pulse rate was significantly steeper in the population from Florida compared to tropical populations or other species. This increase in steepness of female preference facilitated communication of temperate populations in both seasons, indicating an adaptation of female preference to male calls. The evolution of female preference in response to male call plasticity suggests

that developmental plasticity in the communication system complicated the spread of *N. triops* to North America.

Developmental plasticity of male calls

N. triops is the only species of the genus that inhabits both tropical and temperate zones (Greenfield 1990, Walker & Greenfield 1983). It ranges from Ohio in North America to Peru in South America (Whitesell 1974, Walker & Greenfield 1983). Direct egg development and adult diapause in temperate regions indicate a tropical origin of *N. triops* (Whitesell 1974).

In southern parts of temperate North America (e.g. Florida, Texas) male *N. triops* express developmental plasticity in two parameters of their mating calls: (1) At the same temperature, double-pulse rate of summer calls is more than 20% faster than that of winter calls, and (2) summer calls are structured in verses, whereas winter calls are continuous (Whitesell & Walker 1978). These differences between call phenotypes are induced by the photoperiod individuals experience during juvenile development (15 h = summer calls; 11h = diapause & winter calls, Whitesell 1974, Whitesell & Walker 1978).

In the tropics, male *N. triops* only express the summer call phenotype (Greenfield 1990, Walker & Greenfield 1983) with the fast double-pulses and versed call structure. However, if reared under short photoperiods (= 11h light), males from tropical populations expressed significantly slower double-pulse rates (chapter 3). This difference in double-pulse rate of tropical populations was of the same magnitude (>20%) as in temperate populations that do express developmental plasticity in their natural environment. However, males from tropical populations did not change the call structure

(verses vs. continuous) between the call phenotypes, i.e. fast and slow calls were both structured in verses (chapter 2). Thus, tropical populations have the capacity for developmental plasticity of double-pulse rate but not call structure. This finding indicates that developmental plasticity of double-pulse rate and that of call structure have independent developmental and evolutionary pathways. Thus, males do not express the slower double-pulse rate in the tropics because they do not encounter the short photoperiod necessary for its induction.

In the northernmost part of the distribution range (e.g. Ohio), *N. triops* has only one reproductive generation in late spring and males only express the winter call phenotype in their natural environment (Walker 2001, Whitesell 1974). However, it is not known if males still have the capacity for developmental plasticity of calls like the other populations, or if they have lost this capacity. I attempted to investigate this question, but I was not able to find populations of *N. triops* in Ohio or Kentucky. It is possible that I missed these populations because they were reproductively active at a different time of the year (I searched for these population in Ohio and Kentucky end of May and during the first week of June 2007), or populations are very small and occurrence is rather erratic at this end of the distribution range. According to notes of Dr. Walker, the last and only record of *N. triops* found in Ohio dates back to 1956, and records from Indiana and Illinois are from 1950 and 1934, respectively. It is possible that the distribution range of *N. triops* has changed in the last 50+ years and *N. triops* does not occur that far North any more.

We found and heard *N. triops* as far North as Missouri (Poplar Bluff, OMB; Eagle Bluffs, JS) and Tennessee (Dyersburg). According to estimates of Walker (2001) and

Whitesell (1974), populations in Tennessee are expected to be univoltine and produce winter calls. Calls of males that I collected in Dyersburg, TN at the end of April in 2007 were structured in verses and had a mean double-pulse rate of 99.34 ± 4.85 Hz (at 25°C, n = 11), which indicates that males produced summer calls. Dr. Schul heard in August of the same year a population of *N. triops* calling in the vicinity of Dyersburg, TN suggesting that *N. triops* may actually have a bivoltine life history in this geographic region. Overall, the exact distribution pattern, life history, and developmental plasticity of male calls of northern *N. triops* populations are poorly understood and require more research.

Similarly, the most southern populations of *N. triops* offer many opportunities for further research. In the South, the distribution range of *N. triops* extends to Peru (Whitesell 1974) and there are collection records even from Argentina (Systax data base, University of Ulm-Germany). It is conceivable that *N. triops* in temperate South America (e.g. Argentina), like in temperate North America, has two reproductive generations and also expresses different call phenotypes in different seasons. The comparison of features of the communication system of *N. triops* towards the North and the South of the tropics might reveal parallel evolutionary patterns in male calls and female preferences between populations.

Causes for call plasticity

Developmental plasticity of calls seems to have originated in an environment that does not induce its expression (i.e. tropics; chapter 3), female preference had to compensate for the expressed plasticity, and it likely complicated the spread to North

America (see below). Thus, the function and proximate cause of developmental plasticity in *N. triops* remains unclear.

A preliminary experiment gave some insight into the timing and factors influencing the induction of phenotypes. I reared first-instar juvenile *N. triops* at high/low temperatures of 20/28°C for the first three weeks with a photoperiod of 15 h (= long day) and in the fourth week with a photoperiod of 13 h. From the fifth week on I reared animals, like all previous winter generations of *N. triops*, with a photoperiod of 11h (=short day). I transferred adults into diapause for three months at low temperatures (high/low 12/8°C) and short photoperiods (11 h). After breaking of diapause, males were recorded at $25 \pm 1^\circ\text{C}$. Surprisingly, males produced calls with a versed call structure and fast double-pulse rates (average \pm s.d.: 99.13 ± 4.3 Hz, $n = 11$; for comparison: winter calls of lab reared males: 79.1 ± 5.3 Hz; summer calls: 104.3 ± 6.3 Hz & 100.34 ± 3.36 Hz). Both features identified the calls as summer calls. Thus, the long photoperiods (13-15 h) early in juvenile development (first four weeks) seem to have caused the induction of the summer call. This experiment showed that call plasticity is likely not a by-product of physiological changes caused by the diapause conditions (short day length/low temperatures) experienced during winter. Males in this experiment were more than three months older than all other summer males recorded in this study, but still expressed summer calls. This finding corresponds to the results of a previous study that showed that age in winter males did not influence the expression of the call phenotype (Whitesell & Walker 1978).

It is interesting that the decision between phenotypes in *N. triops* takes place within the first half of juvenile development. This relatively early developmental decision

may indicate that the physiological changes necessary for the expression of the summer phenotype (e.g. summer call, immediate development of female ovaries and male accessory glands; Whitesell 1974) and the winter phenotype (e.g. suppressed development of ovaries and accessory glands, production of fat tissue as preparation for diapause; Whitesell 1974) are fairly substantial and possibly require long developmental times. Further rearing experiments in which light/dark cycles are systematically manipulated could determine the length of the critical photoperiod that decides between the expression of summer and winter call phenotype and when the decision exactly takes place.

Developmental plasticity might be an epiphenomenon related to the aforementioned physiological changes. It is also possible that the reduction of double-pulse rate in the winter call is the result of a pleiotropic gene effect, i.e. the expression of genes that are responsible for adaptive changes in other traits may also influence features of calling such as the movement speed of muscles and therefore the double-pulse rate of the winter call.

However, further experiments are needed to understand the causes of call plasticity. *N. triops* is the only species of its genus that lives in both the tropics and temperate regions (Greenfield 1990). Developmental plasticity originated in the tropics but was only expressed when *N. triops* experienced new environmental conditions after the spread to North America. Rearing experiments with other species of *Neoconocephalus* may induce similar changes of male calls in response to unusual environmental conditions or photoperiods. These experiments may reveal developmental plasticity that is usually hidden in other species due to a lack of environmental variation.

Induced variation of calls in other species would indicate a common (genetic and/or physiological) basis for call plasticity and may further suggest that plasticity in *N. triops* is rather an epiphenomenon than a specifically evolved trait.

Temperature coupling

Many species of *Neoconocephalus* (including *N. triops*) produce calls with exceptionally fast pulse rates: 21 out of 25 described species produce calls with rates well above 100 Hz, some species even extend beyond 200 Hz (Greenfield 1990). Most insects and anurans emit calls with pulse rates between 10 and 80 Hz (Gerhardt & Huber 2002). Male *Neoconocephalus* use synchronous muscles that drive the wings for stridulation (Heath & Josephson 1970). In order to produce these fast movements, males of fast calling species have to warm up prior to calling and produce heat during calling. As a result, males may elevate thorax temperature up to 15°C above ambient temperature (Heath & Josephson 1970, Josephson 1984). The increase in thoracic temperature is larger for lower ambient temperatures, so that thorax temperature during calling is almost constant (Heath & Josephson 1970).

This elevated temperature of fast calling *Neoconocephalus* leads to a weak dependency of pulse rates on ambient temperature (Walker 1975). In average, calls of *Neoconocephalus* males change by 3.13 %/°C (n = 8 species; relative to rate at 25°C; data from Büttner 2002, and my call recordings). In comparison, crickets of 5 subfamilies change by 4.83 %/°C (n = 19, Walker 1969b) and katydids of the genus *Orchelimum* change by 7.17 %/°C (n = 12, Walker 1975).

Calls and preferences for calls should be matched for communication systems to function. Because (silent) females do not produce heat, the reduced temperature dependency of fast *Neoconocephalus* calls should complicate the temperature coupling between calls and preference over a wide temperature range. However, in the three species of *Neoconocephalus* tested, communication remained functional between 22°C and 29°C (Fig. 1). *N. affinis* and *N. bivocatus* both use double-pulse rate for call recognition (Bush et al. in prep., Deily & Schul 2004) and *N. robustus* uses interval duration (Deily & Schul 2004). Temperature dependencies were similar between female preference (*N. affinis*: 3.10%/°C, *N. bivocatus*: 3.88 %/°C) and male calls (3.61 %/°C and 4.32 %/°C, respectively) facilitating each communication system to remain functional over a wide range of temperatures (Fig. 1). In *N. robustus*, female preference and male calls had a different temperature dependency (preference: 4.10 %/°C, calls: 2.27%/°C), but the communication system remained functional due to the different call recognition mechanism: interval durations accepted by females were much longer than the intervals produced by male calls at the same temperature (Fig. 1).

Populations of *N. triops* in North America express two substantially different calls and populations in the tropics only one. In Costa Rica female preference and male summer calls have a similar temperature dependency, facilitating communication over a wider range of temperatures. In Florida, female preference is much steeper (Chapter 4, see below) than male calls, resulting in an overlap of female preference and each male call type only in a narrow range of temperatures. However, this steeper temperature dependency of female preference is necessary to match female preference to two substantially different calls in the summer and winter (chapter 4).

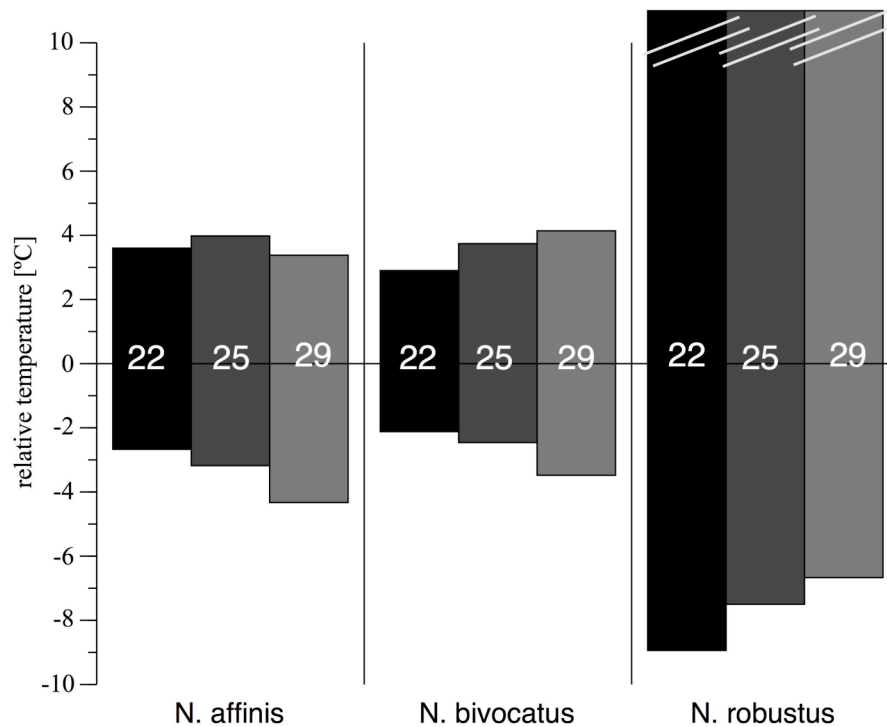


Fig. 1

Temperature coupling between female preference and male calls in species of *Neoconocephalus*. For each species, we determined the pulse rate range the elicited strong phonotaxis (score ≥ 0.7) in females at a given temperature. We calculated from regression lines of pulse rates of calls (Büttner 2002) the corresponding temperature range of male calls that was attractive for females. Thus, the temperature indicated on the y-axis indicates how much warmer or colder the male call can be to still elicit strong responses at a given temperature of the female (22, 25, or 29°C). Note that *N. robustus* uses interval duration for call recognition, i.e. calls are attractive if the interval duration is shorter than a critical value (Deily & Schul 2004). Since intervals decrease in duration with temperature, the match between calls and preference was not limited towards higher temperatures.

Female preference for male call traits

Developmental plasticity of calls causes changes in two call parameters: double-pulse rate and call structure (Whitesell & Walker 1978). Males from both temperate and tropical populations have the capacity for developmental plasticity of double-pulse rate, whereas only temperate populations have the capacity for plasticity in call structure (chapter 3).

Females of both temperate and tropical populations have strong preferences for double-pulse rate and use this parameter for species identification (chapter 2). However, the shape of female preference for double-pulse rate differs between populations. Preferences of females from Florida and Puerto Rico are 'closed', i.e. only a certain range of double-pulse rates elicits strong responses and responses decrease towards faster and slower rates at both 20 and 25°C (Fig. 2). In the population from Costa Rica however, female preference is only 'closed' at 20°C but 'open' at 25°C, i.e. attraction decreases only towards slower double-pulse rates but not faster rates (Fig. 2). This open shape of female preference at 25°C resembles the preference functions of *N. robustus* (chapter 4), a species that uses interval duration for call recognition (Deily & Schul 2004). Call recognition based on interval duration likely represents an ancestral state in *Neoconocephalus* (Deily 2006). Thus, preference of females from Costa Rica seems to shift *functionally* with temperature between the derived state (rate recognition at 20°C) and ancestral state (interval duration recognition at 25°C) of call recognition. It remains unclear if the shape of female preference at 25°C is the result of a call recognition mechanism that is actually based on interval duration or represents an epiphenomenon of

rate recognition in response to the exceptionally fast pulse rates of this population (=130 Hz double-pulse rate at 25°C, Fig 3).

Only versed calls elicited strong responses in Costa Rica females but not continuous calls. In contrast, females from Puerto Rico and Florida are strongly attracted to both continuous and versed call models (Fig. 4). Thus, females from Costa Rica differ in their preference for call structure from other populations of *N. triops*.

N. punctipes co-occurs with *N. triops* in Costa Rica and produces continuous calls with pulse rates comparable to *N. triops* (~ 250 Hz single-pulse rate at 25°C, Walker & Greenfield 1983). At 25°C, *N. triops* females from Costa Rica have open preferences for pulse rate (Fig. 2), likely not preventing attraction to pulse rates of *N. punctipes* calls. Thus, the strong preference for versed calls of females from Costa Rica may be necessary to preserve species identification in Costa Rica.

In Puerto Rico, the only co-occurring *Neoconcephalus* species with similar pulse rates is *N. maxillosus* (~130 Hz double-pulse rate at 25°C; Walker & Greenfield 1983, and Fig. 3). However, female *N. triops* are not strongly attracted to these fast rates (phonotaxis score = 0.4, Fig. 2B) and *N. maxillosus* is active later at night than *N. triops* (personal observation). In Florida, the species with the most similar pulse rate is *N. retusus* (~ 90 Hz double-pulse rate at 25°C, Büttner 2002). But again, female attraction of *N. triops* at this rate is low (phonotaxis score = 0.34, Fig. 2A) and *N. retusus* is reproductively active later in the summer than *N. triops* (Walker 2001). Thus, selectivity of call structure is not necessary for species identification in Florida and Puerto Rico, possibly explaining the reduced selectivity for this parameter in these populations. This change of selectivity may have enabled the evolution of developmental plasticity of call

structure (versed summer calls and continuous winter calls) in the population from Florida.

Female preference for double-pulse rate also differs in its temperature dependency between populations (Chapter 4). Temperature dependency of female preference changed the least for females from Costa Rica (4.10 %/°C, relative to 25°C) and the most for females from Florida (5.82%/°C). Change of preference of Puerto Rico females was intermediate compared to the other two populations (4.90%/°C). Female preference of other *Neoconocephalus* species changed by approximately 3.80 %/°C (chapter 4), indicating that the temperature dependency of the population from Puerto Rico is steeper than expected.

Female preference from populations of Florida and Puerto Rico resemble each other in several aspects: Female of both populations are strongly attracted to both versed calls and continuous calls, temperature dependency of preference of double-pulse rate is steeper than in other populations or species, and preference for pulse rate remains closed at 25°C (Fig. 2). All these aspects of female preference differ to the features of preference exhibited by females from Costa Rica (see above). These similarities could indicate gene flow between populations from Florida and Puerto Rico, or could suggest a temperate origin of the Puerto Rico population. However, males from Puerto Rico (like males from Costa Rica) lack the capacity for developmental plasticity of call structure, contradicting this hypothesis. Thus, only a study of the population genetics of this species could clarify gene flow and the relationship between populations.

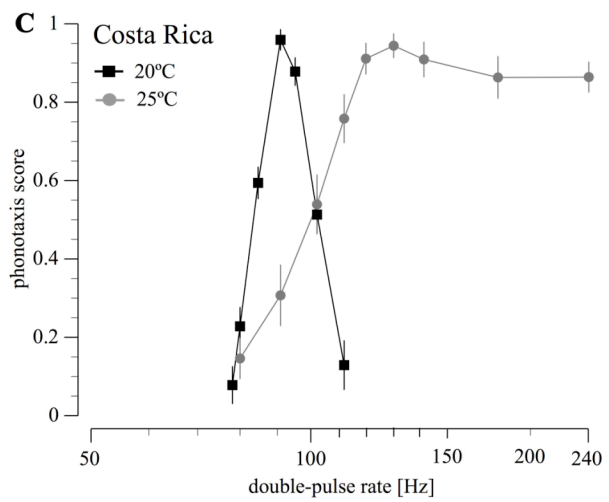
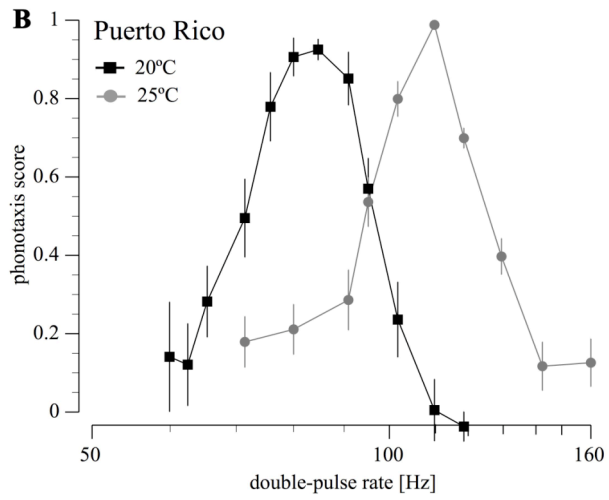
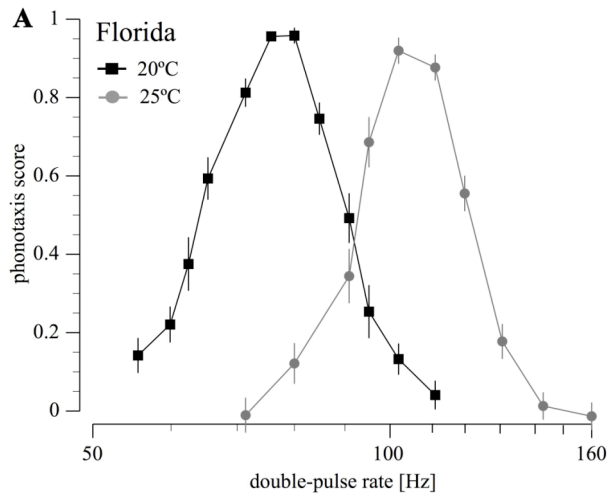


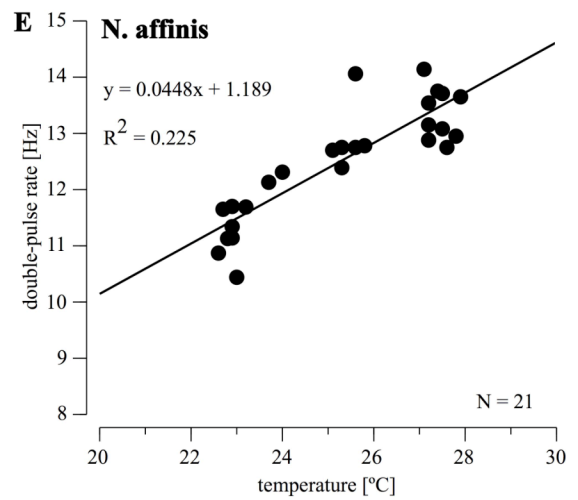
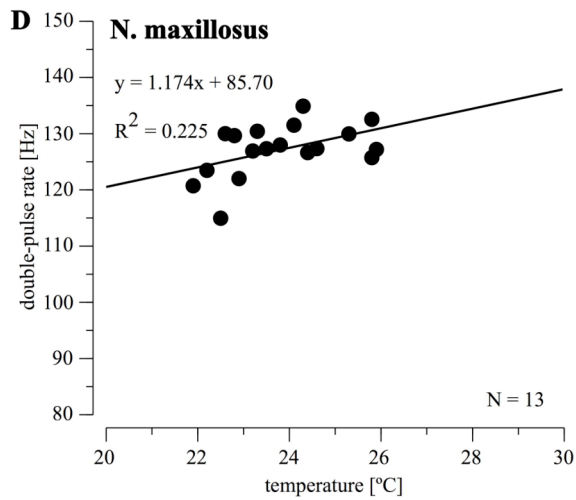
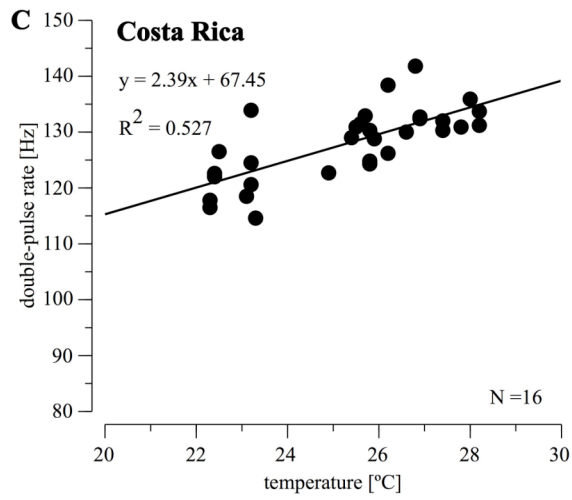
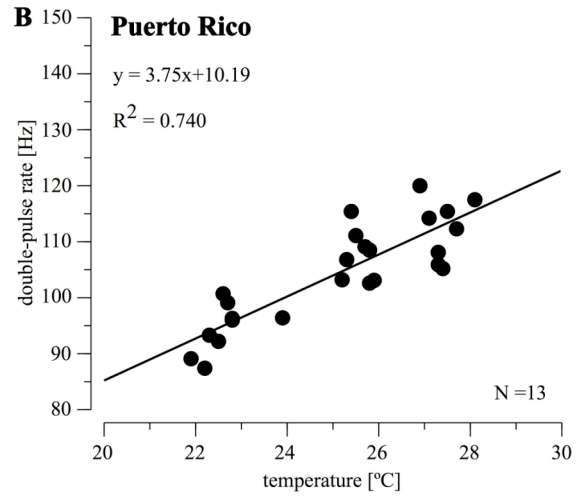
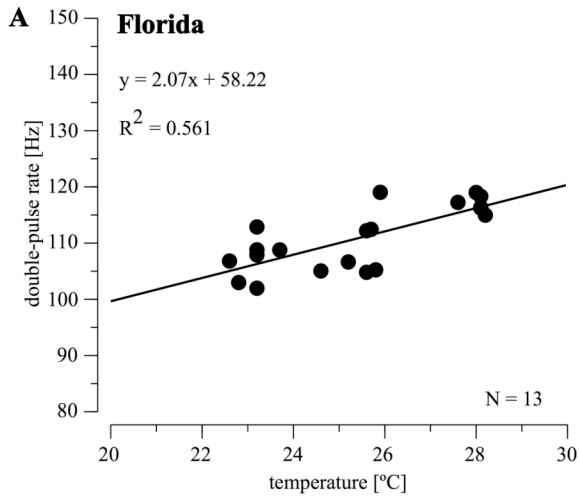
Fig. 2 (previous page)

Response functions of female *N. triops* from Florida (A), Puerto Rico (B), and Costa Rica (C) at 20°C (black) and 25°C (grey) in response to double-pulse rates. Response functions of each female were smoothed by calculating a gliding averages over three values and then normalized. Each symbol indicates the average phonotaxis score (\pm s.e.m.) for a given double-pulse rate. Female preference did not differ between summer and winter *N. triops* from Florida (chapter 2). Therefore, data shown in (A) is based on pooled data from both generations. Number of females tested: Costa Rica: 20°C =10, 25°C =10, Puerto Rico: 8/9, Florida: 16/19, respectively.

Fig. 3 (next page)

Temperature dependency of double-pulse rate of summer calls of *N. triops* from (A) Florida, (B) Puerto Rico, (C) Costa Rica, (D) *N. maxillosus*, and (E) *N. affinis*.

We recorded male calls with a custom-made A/D converter system (16-bit resolution, 250 kHz sampling rate) and ¼" electret microphones (RadioShack 33-3028, frequency response 30 to 18,000 Hz). For each male we calculated the mean AM-rate of at least 100 consecutive double-pulses. We calculated linear regression lines of AM-rates for each population or species by reducing the sum of error squares (regression formula: top left corner). Each male contributed one or two AM-rates to the regression line (N = number of recorded males; bottom right).



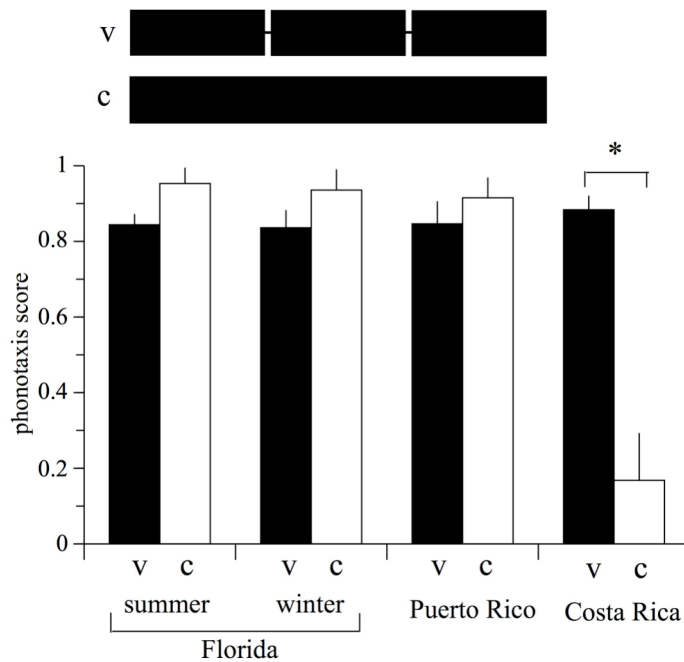


Fig. 4

Mean phonotaxis scores (\pm s.e.m.) of female *N. triops* from different populations to calls structured in verses and continuous calls. (Top) Schematic representation of stimuli with verse structure (v) and continuous call (c). (Bottom) Mean phonotaxis scores (\pm SEM) of females from Florida (summer generation: $n = 11$, winter generation: $n = 10$) Puerto Rico ($n = 9$), and females from Costa Rica ($n = 10$) to versed call models (v) and continuous call models (c). The stimuli for the experiments consisted of merged pulses or double-pulses that were interspersed by silent gaps. Versed stimuli for experiments with summer and winter females from Florida and Puerto Rico: train of paired pulses of 2 ms and 2.75 ms duration with an interval of 1.75 ms in between. These double-pulses were repeated after an interval of 2.5 ms, resulting in a double-pulse rate of 111 Hz. Versed call for experiments with females from Costa Rica: double-pulses were merged to one long pulse with an duration of 6.0 ms and intervals between pulses of 2.4 ms (= 119 Hz). Pulse

structure of continuous call for summer females from Florida: same as for versed call (see above); winter females from Florida and females from Puerto Rico: double-pulses were merged to long pulses with a duration of 6.5 ms and intervals between pulses of 2.5 ms (= 111 Hz); Costa Rica females: same as versed call (see above). The versed stimulus used for experiments with females from Florida & Puerto Rico had verse durations of 950 ms and interval durations of 50 ms and that for experiments with Costa Rica females 650 ms and 50 ms, respectively (values based on call recordings of summer males from Costa Rica at 25°C). Phonotaxis scores to continuous and versed calls were not significantly different from each other in both generations of the Florida population and the population from Puerto Rico (Signed rank Wilcoxon-tests for Florida: $0.1 > p > 0.05$ for both generations; Puerto Rico: $0.5 > p > 0.2$). Phonotaxis scores for versed and continuous stimuli differed significantly (indicated by asterisk) for females from Costa Rica (Signed rank Wilcoxon-tests for Florida: $p < 0.001$). All females were tested at 25°C ambient temperature.

Ecological and evolutionary aspects of developmental plasticity

Developmental plasticity can play an important role in the context of ecology.

The ability to express alternative phenotypes in response to changes in the environment can be adaptive for survival in recurrently changing environments (Yeh & Price 2004, Trussel & Smith 2000, Laurila et. al 2002). Developmental plasticity can also facilitate the spread to new environments and therefore contribute to the extension of the distribution range of species (Yeh & Price 2004, Ehrlich 1989, Holway & Suarez 1999).

In *N. triops*, however, developmental plasticity did not function in the ecological contexts mentioned above. On the contrary, the expression of substantially different call phenotypes in the different seasons of North America even required a change of female preference to compensate for these differences. Thus, developmental plasticity of male calls in *N. triops* did not facilitate but rather hindered the spread of *N. triops* to North America. This finding emphasizes the possible complications related to the spread of organisms into new environments. It is not only necessary to have physiological traits allowing the survival under the new conditions, but also reproduction itself has to be ensured for a successful establishment in a new environment. *N. triops* is the only species of its genus that inhabits both temperate and tropical regions ranging from Ohio to Peru (Walker & Greenfield 1983). If female preference had not adapted to male call plasticity, *N. triops* would probably not have today's impressive ecological success.

Developmental plasticity has been described as a potent initiator of evolutionary change in two contexts (review in West-Eberhard 2003): (1) Plasticity can generate phenotypic variation in response to changes in environmental conditions (Pigliucci et al. 2006, Agrawal 2001). If the induced variation has a fitness effect and a genetic basis,

selection will act on it, ultimately leading to evolutionary genetic change (West-Eberhard 2003). (2) Developmental plasticity itself is a trait with a partial genetic basis and can evolve (West-Eberhard 1989, Agrawal 2001, Pigliucci 2005). Our results suggest another way how developmental plasticity can lead to evolutionary change. Plasticity expressed in one trait can impose a selection pressure on a related trait and thus lead to the evolution of the trait: in *N. triops* expression of male call plasticity selected for changes in the temperature dependency of female preference.

Conclusions

Plasticity of mating calls seems to be an ancestral trait of *N. triops* and the spread to North America exposed it to conditions inducing its expression. Overall, the expression of developmental plasticity influenced the evolution of the communication system of *N. triops* in unexpected ways. Call plasticity was not accompanied by a similar developmental plasticity of female preference that could have preserved the match between female preference and male calls. Instead, female preference increased its temperature dependency to match the seasonal variation of male calls in two different temperature ranges. Considering that in most communication systems female preference usually drives the evolution of male calls, the finding that female preference adapted to male calls in *N. triops* was rather surprising. Moreover, the expression of seasonally different calls in North America required this change of female preference, indicating that call plasticity likely complicated the spread of *N. triops* from the tropics to North America.

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Appendix

(Electronic supplementary material of 2. chapter)

Importance of pulse structure for female phonotaxis in *N. triops*

We tested attraction of summer females to stimuli with different pulse structures (Fig. 1): stimuli with the natural double-pulse pattern (control), a stimulus without amplitude modulation (sine), and merged double-pulses (merged). All stimuli had the summer-specific verse structure (verse duration: 950 ms, interval duration: 50 ms) and a carrier frequency of 11 kHz. Phonotactic response to unmodulated stimulus (sine) was significantly lower than response to control stimulus (asterisk, Wilcoxon signed-rank test, $p < 0.005$); phonotactic response to merged pulses was not significantly different from the response to the control (Wilcoxon signed-rank test, $0.5 > p > 0.2$). These results indicate that some amplitude modulation is required for call recognition. However, the detailed double-pulse pattern is not required, as one long pulse in lieu of the double-pulse is equally attractive.

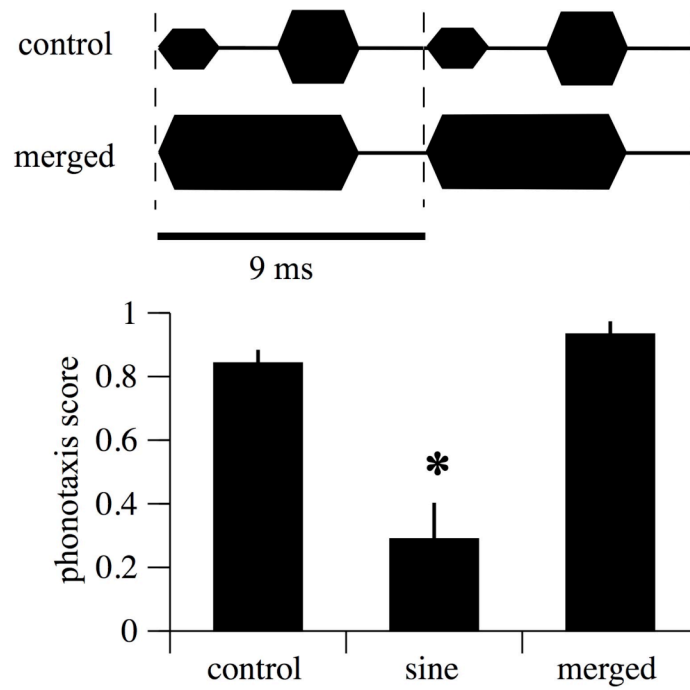


Fig. 1

(Top panel) Schematic representation of control stimulus and stimulus with merged pulses. (Bottom panel) Mean phonotaxis scores (\pm sem, $n=12$) of summer females to stimuli with the natural double-pulse pattern (control), a stimulus without amplitude modulation (sine), and merged double-pulses (merged).

Importance of pulse and interval duration for female phonotaxis in *N. triops*

We tested phonotactic responses of summer females of *N. triops* to stimuli that varied in (merged) pulse duration and interval duration (Fig. 2). All stimuli had a carrier frequency of 11 kHz and were continuous. Females showed significant phonotaxis to stimuli when the pulse period (i.e. the sum of pulse duration and interval duration) was close to 9 ms, i.e. an AM-rate of 111 Hz, largely independent of pulse and interval duration. These responses appear in the response field along a diagonal from bottom right to top left. Towards higher and lower pulse rates, response magnitude decreased sharply. Thus, the AM-rate rather than the pulse duration or interval duration was the critical parameter evaluated by female *N. triops*.

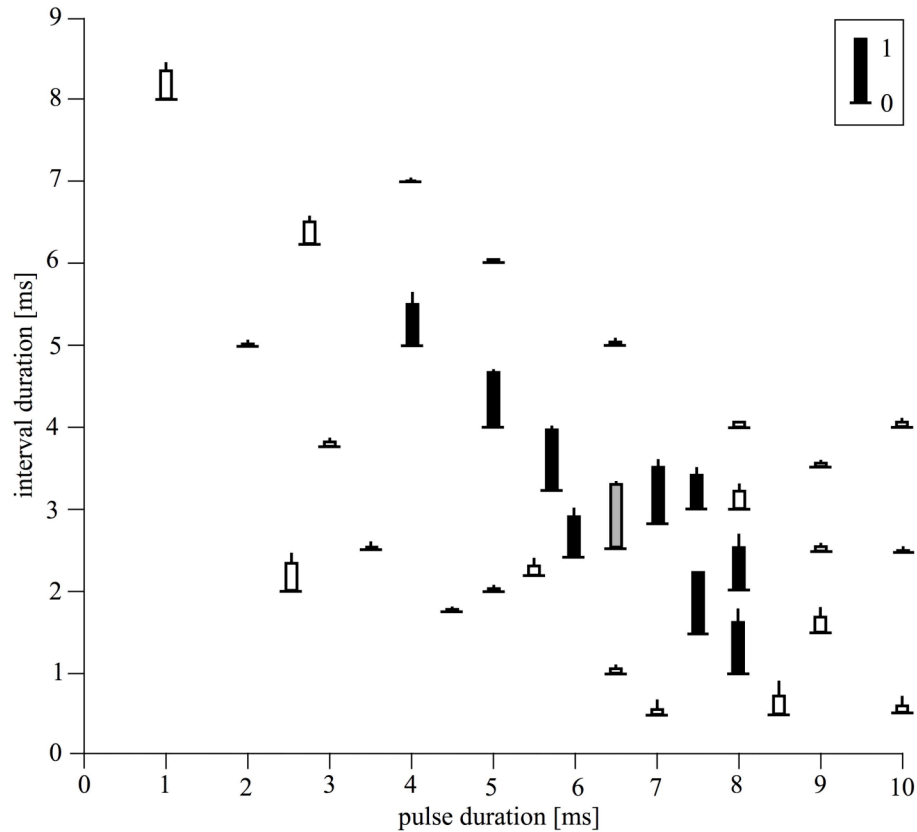


Fig. 2

Phonotactic responses of females to stimuli that were varied in pulse duration and interval duration. Bars indicate mean phonotaxis score (+ sem, n = 6-14) for each parameter combination. The baseline of each bar is positioned on the interval duration of the stimulus. Filled bars indicate significant responses, i.e. phonotaxis score was significantly greater (Wilcoxon paired-sample test, $p < 0.05$) than the mean score from the same females in response to silence, and the average score was at least 50% of the score to the control. Open bars indicate non-significant responses, and the gray bar indicates responses to the stimulus with natural parameter combination if double-pulses are merged. Inset serves as a scale for the phonotaxis

VITA

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