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The effect of temperature on sex determination

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Summary and Conclusions

Temperature is the result of the motion of molecules, and increases as the molecules move faster. As organisms are largely made of molecules, this simple principle has important implications on all levels of biological organization. From simple chemical reaction rates, via individual organisms, to entire ecosystems, all are affected by temperature one way or another (Johnston & Benett, 1996). This thesis is concerned with one specific effect of temperature, namely on the sexual development of animals.

In many cold blooded species, like many reptiles and fish, the sex of an individual is determined by temperature during embryogenesis with some temperatures leading to female and others to male development. This is called temperature dependent sex determination (TSD). For example in turtles, high incubation temperatures lead to female and low temperatures to male development. In crocodiles the opposite pattern is observed with high temperatures resulting in male and low temperatures in female development. In contrast, in most other organisms, such as all mammals and birds, the sex of an individual is determined by its genotype. Such a system is called genetic sex determination (GSD). Recent work on the molecular basis of sex determination has revealed that the dichotomy between GSD and TSD may not be as sharp as previously believed. The underlying gene networks are quite similar and small changes can convert a GSD system into TSD and vice versa. In addition, more and more species are being discovered where both genes and temperature affect sexual development, and where multiple sex determining (SD) systems coexist within one species. Phylogenetic analyses strongly indicate that multiple transitions between GSD and TSD, in both directions, have occurred. Finally, the geographic distribution of sex determining mechanisms also follows temperature gradients, TSD being confined mostly to warmer regions of the globe, and different types of GSD sometimes follow geographical clines as well. These evolutionary and geographical patterns are still far from being well-understood, and it is the goal of this thesis to bring us closer to such an understanding.

In general, the effects of temperature on sex determination can be classified in two categories. On the one hand temperature can be viewed as proximate trigger that directly affects organisms themselves, such as in TSD. On the other hand temperature can be seen as ultimate cause, setting the selective environment for

the evolution of and transition between different sex determining systems, thereby also affecting the geographical distribution of sex determining systems in nature. In this thesis the main emphasis lies on the second category with the focus on the ultimate effect of temperature leading to the transition between different sex determining systems and their distribution. However, ultimate issues cannot properly be addressed without considering the underlying proximate mechanisms as well.

This thesis is divided into two parts. In the first part a modeling approach is taken to address questions about evolutionary transitions between GSD and TSD and the conditions which favor coexistence of different SD systems. However, this part is not entirely separated from reality, since some of the models are parameterized with empirical data from the snow skink, *Niveoscincus ocellatus*. The second part of this thesis is concerned with empirical research on the housefly, *Musca domestica*, a global species with geographical clines in frequencies of different GSD systems. With experiments and fieldwork a suite of hypotheses regarding the origin of these geographical distributions were systematically tested.

Part I: Theoretical modeling approach

There are several hypotheses regarding the evolution of TSD from GSD (Ewert & Nelson, 1991; Shine, 1999), but by far the most influential hypothesis is embodied in the model of Charnov and Bull (1977). The basic idea is that the fitness of males and females is differentially affected by variation in environmental conditions. Specifically, under some conditions females have higher expected fitness than males, while other conditions benefit males more. TSD is a mechanism that allows for flexible adjustment of sex ratios in situations where biased sex ratios are selectively favored. GSD does not readily allow for such flexible facultative sex ratio adjustments, thus giving TSD a selective advantage in these cases. However, the very flexibility of TSD can also be a liability when fluctuations in temperature between years are sufficiently large. In that case, years with extreme temperatures may lead to very biased sex ratios among the offspring, and thereby cause (near) population extinction. This is less of a problem in long-lived species, where the fluctuations over a lifetime tend to cancel each other out.

In **Chapter 2** a model is presented that does not assume environment dependent sex-specific fitness effects, but is based on selection for unifactually biased sex ratios due to local kin competition. Sex ratio theory predicts that the more dispersive sex should be overproduced since it is least likely to compete with relatives for resources or space. The basic idea is that under these conditions TSD is likely to evolve because it allows for unifactually biased sex ratios and therefore has an advantage over GSD. In the model the degree of environmental fluctuations and longevities was systematically varied to understand the interplay and importance of the different factors. Starting with a GSD population it was investigated whether and under which conditions TSD might evolve. Five different outcomes were observed: (i) the initial GSD state was stable; (ii) TSD evolved and took over; (iii) a new type of GSD evolved at a second locus; (iv) GSD and TSD stably coexisted; (v) multiple coexisting GSD systems evolved. In line with earlier work, TSD evolved more easily when environmental fluctuations were small and when lifespan was long. In the simulations rapid evolutionary transitions between GSD and TSD were common even without the assumption of temperature dependent sex-specific differential fitness, thus offering a new, additional explanation for the evolution of TSD. Another important insight from the model is that multiple sex determining systems can evolve from identical initial conditions, which may be governed by random factors such as genetic drift.

Although conceptual models as in Chapter 2 are important to gain general insights into the evolution of TSD, more specific models tailored to specific species are needed to test these insights. Most species are unsuitable to test hypotheses on the evolution of TSD because they possess a single sex determining system. The snow skink *Niveoscincus ocellatus* is an exception since both GSD and TSD populations exist within this species in different geographical regions. Moreover, a large amount of knowledge about the life history of this species has accumulated over the years. Females in the TSD population that are born early during the breeding season have a higher probability of reproducing one year earlier compared to late born females. In the GSD population the probabilities of breeding early still differ between early and late born females, but to a lesser extent. In both populations male fitness is independent of birth date. While early and late born females in the GSD population show fitness differences, large between-year temperature

fluctuations in their range might lead to a selective advantage of GSD. In **Chapter 3** models parameterized with data from the skinks life history and climatic data were used to predict which SD system would evolve in the different skink populations. The results show that the model correctly predicts the sex determining system for each population based on local temperature variation and demography of the skinks.

Three main conclusions can be drawn from this theoretical part of the thesis. First, selection for unfacultatively biased sex ratios can lead to transitions between GSD and TSD, even without assuming temperature dependent sex-specific fitness effects. These results might offer a new explanation for the evolution of TSD and might explain why several studies failed to find sex specific fitness effects in certain species. Second, the simulations did not only result in transitions from GSD to TSD but also in multi-factorial GSD, and coexistence of GSD and TSD, hence providing a potential explanation for observed instances of coexistence. Third, tailoring an evolutionary model to a specific species can be useful to make predictions on the evolution of sex determining systems in this species.

Part II: Empirical Approach

In the second part of this thesis the main goal was to study the effect of temperature variation on the distribution of sex determining (SD) factors in the housefly, which was investigated by a series of experiments and statistical analyses. The default sex of houseflies is female since all individuals carry the female determining factor F . Individuals that carry an additional male determining factor M , which suppresses the function of F , turn into males. In the so called standard XY system, which is most common at high latitudes and high altitudes, M is found on the Y chromosome. In populations at lower latitudes and altitudes the M factor can be found on any, or even multiple of the five autosomes. In populations with autosomal M factors females often possess a mutated version of F , the F^D factor, which is insensitive to M .

But what causes these clines? The most obvious factor that varies along latitude is temperature. This led several authors to suggest that the distribution of SD factors is somehow caused by variation in temperature (Franco *et al.*,

1982; Çakir & Kence, 1996); to what extent this is the case is the subject of this part of the thesis.

A simple hypothesis that could explain the distribution of SD factors is that temperature affects the fitness of flies, in a way that depends on their sex determining factors. Therefore the goal of **Chapter 4** was to experimentally investigate whether houseflies with autosomal M or F^D have higher fitness at higher temperatures compared to standard XY flies. To determine whether autosomal M factors could invade the standard XY population, some males with the M factor on autosomes II and III were introduced into standard XY populations under different temperatures, and their frequency was tracked over several generations. The results were less straightforward than expected. Although M on autosome II replaced the Y, M on autosome III did not increase in frequency and no effect of temperature was detected. To compare the fitness of females with and without F^D , several fitness parameters of female flies with either sex determining factor were measured at different temperatures. There was great variation between populations, but again, as in the male experiment, no effect of temperature was detected.

In the previous chapter none of the investigated fitness parameters showed an influence of temperature. For **Chapter 5** the goal was to study an additional fitness component, the frequency of intersexes (low fitness individuals possessing both male and female characteristics) among offspring. It has been reported that the frequency of intersexes in the housefly increases in winter (Milani, 1967), which lead to the hypothesis that their frequency increases at cold temperatures. Therefore intra- and inter-population crosses with standard XY as well as autosomal M populations with different frequencies of F^D were conducted and distributed over different temperatures. For each of the crosses offspring were examined for intersex characteristics. No effect of temperature on the frequency of intersexes at any of the temperature treatments was detected. However, population sex ratios were affected by temperature in some of the experiments. This suggests that sexual development might be influenced by temperature but further experiments are needed to investigate this more closely.

Another way of investigating the dynamics of and selection on housefly SD factors besides studies in the laboratory over short time spans is to study long term changes in distribution patterns in the field. Autosomal M factors were first reported some 50 years ago, and it was hypothesized that they are spreading northward, replacing the standard XY system. **Chapter 6** reports on the collection of houseflies from populations across Europe, that had been studied 25 years ago (Franco *et al.*, 1982), in order to investigate how and if the distribution had changed over time. In contrast to earlier predictions no clear change in the distribution of autosomal M factors in comparison with the distribution 25 years ago was detected. This implies that autosomal M factors do not have a uniform advantage over the standard M factor, since otherwise it would have completely replaced the latter. The apparent stability of the cline suggests that XY has an advantage in the north, while autosomal M factors seem to have an advantage in the south.

For **Chapter 7** the goal was to further investigate the proposition that the geographical distribution of housefly SD factors is caused by variation in temperature. If this is indeed the case one would expect to find similar distribution patterns in the southern hemisphere compared to the once found in the north. Therefore samples were collected from several locations in South Africa and Tanzania. The results show that housefly populations on the southern hemisphere repeat the pattern earlier found on the northern hemisphere, higher frequencies of autosomal M and F^D factors are observed closer to the equator or lower altitudes.

Not only temperature but also other climatic variables change systematically along latitudinal and altitudinal clines. Nowadays climatic databases are publically available, making it possible to obtain data on a number of climatic factors on a global scale. Using the newly collected data on SD factor frequencies of the southern hemisphere plus data of previously published studies and data on several climatic variables a meta analysis was conducted to investigate which climatic variable(s) can best explain the clinal distribution of the SD factors. The results show that seasonality in the degree of temperature variation rather than mean temperature best explains the distribution of the male SD factor, in the sense that autosomal M factors are more common in places with less seasonal temperature fluctuation. For female SD factors on the other

hand the combination of humidity and yearly mean temperature best explains their distribution, low humidity and high mean temperature being positively correlated with the frequency of F^D .

For **Chapter 8** the main goal was to extend the available molecular tools for studying the housefly by developing new microsatellite markers and constructing a linkage map. The markers mapped to five linkage groups, corresponding with the five autosomes of the housefly, but none of the markers mapped to either the X or the Y chromosome.

Theory on sex chromosome evolution predicts that recombination rates on chromosomes acquiring sex chromosome function should decrease along the chromosome starting from the location of the sex determining gene. The reason for this is, that genes accumulate on the Y chromosome for example, which are beneficial for males, however disadvantageous for females. Reduced recombination rates prevent these genes from ending up in females. For the housefly this implies that recombination rates on autosomes containing the M or F factor should also show a decrease in recombination rates compared to autosomes without. To test this prediction, recombination rates of autosomes containing a SD factor were compared with autosomes without, by making use of markers of the specific autosomes based on the results of the linkage analysis. A statistical analysis revealed significantly reduced recombination rates on autosomes with SD factors, in line with theoretical expectations.

Given our results of the housefly experiments can we now claim that we understand the geographical distribution of the sex determining factors and the role of temperature? On the one hand the finding that the distribution of SD factors in the southern hemisphere follows the patterns found in the north hints towards a role of temperature in shaping these clines. On the other hand experiments failed to show an effect of temperature on the fitness of houseflies with different SD factors. This is perhaps not too surprising in the light of the subsequent meta analysis, which suggests that not temperature per se but rather seasonality or the combination of humidity and mean temperature might be responsible for the clinal distribution of SD factors. Whether the correlations from the meta analysis reflect causal effects remains to be investigated in future experiments.