Genetic adaptation in emergence time of *Clunio* populations to different tidal conditions

DIETRICH NEUMANN

Zoologisches Institut der Universität Würzburg, Würzburg

KURZFASSUNG: **Genetische Adaptation der Schlüpfzeiten von *Clunio*-Populationen an verschiedene Gezeitenbedingungen.** Die Schlüpfzeiten der in der Gezeitenzone lebenden *Clunio*-Arten (Diptera, Chironomidae) sind mit bestimmten Wasserstandsbedingungen synchronisiert, und zwar derart, daß die unmittelbar anschließende Fortpflanzung der kurzlebigen Imagines auf dem trockengefallenen Habitat stattfinden kann. Wenn das Habitat einer *Clunio*-Art in der mittleren Gezeitenzone liegt und parallel zu dem halbtägigen Gezeitenzyklus (T = 12,4 h) zweimal täglich auftaucht, dann kann sich eine 12,4stündige Schlüpfperiodik einstellen (Beispiel: *Clunio takahashii*). Wenn das Habitat in der unteren Gezeitenzone liegt und nur um die Zeit der Springtiden auftaucht, dann ist eine 15tägige (semilunare) Schlüpfperiodik zu erwarten (Beispiele: *Clunio marinus* und *C. mediterraneus*). Diese 15tägige Schlüpfperiodik ist synchronisiert mit bestimmten Niedrigwasserbedingungen, die an einem Küstenort alle 15 Tage jeweils um die gleiche Tageszeit auftreten. Sie wird daher durch zwei Daten eindeutig gekennzeichnet: (1) die lunaren Schlüpftage (wenige aufeinanderfolgende Tage um Voll- und Neumond) und (2) die tägliche Schlüpfzeit. Wie experimentelle Untersuchungen über die Steuerung der Schlüpfperiodik zeigten, können die Tiere beide Daten richtig vorausbestimmen. Die einzelnen Küstenpopulationen unterscheiden sich allerdings in Anpassung an die örtlichen Gezeiten- und Standortbedingungen recht auffällig in ihren lunaren und täglichen Schlüpfzeiten. Kreuzungsversuche zwischen Laboratoriumsstämmen verschiedener Populationen belegen, daß die Unterschiede in der täglichen Schlüpfzeit genkontrolliert sind.

INTRODUCTION

In the field, organisms must be able to tolerate all immediate fluctuations of the environmental conditions and must further be able to synchronize their development and behavior with the periodic fluctuations of these conditions. Adaptations to the cycle of day and night and to the cycle of seasons are, therefore, common both in aquatic and terrestrial organisms. Intertidal organisms are additionally exposed to the cycle of tides. The semidiurnal cycle of tides has a period of 12.4 hours; consequently, both low and high tide are generally 50 min later each day, occurring only every 15 days at about the same time of day (Figs. 1 and 2). Besides this semidiurnal cycle, the tidal range at many coasts shows a semimonthly (semilunar) cycle of spring and neap tides: spring tides periodically occur 1 to 2 days after full and new moon, neap tides 1 to 2 days after half moon. Rhythmic adaptations to these two cycles of tides are known to occur in many animals. Such adaptations include tidal rhythms of movement, locomotion and color change, and semilunar and lunar rhythms of breeding (CASPERS 1951, KORRINGA 1957). A small number of laboratory studies has given insight into the mechanisms and control of these rhythms (HAUENSCHILD 1960, BÜNNING & MÜLLER 1961, ENRIGHT 1963, VIELHABEN 1963, NEUMANN 1966). In this paper I will report observations and experiments with the intertidal midge *Clunio*.

RESULTS AND DISCUSSION

Clunio was first described from the Irish and English coasts more than 100 years ago, and several species from European and Pacific coasts have since been recognized. The imagines are marked by a striking dimorphism of sexes: the male is winged (length of *C. marinus* ca. 2 mm) while the female is wingless. Their biology is characterized by the following details: (1) the larvae settle on a specific substrate which occurs only over a narrow range in the intertidal zone, (2) the eggs have to be deposited on the exposed habitat, (3) the short-lived imago can only reproduce within 2 hours after emergence. The times of emergence and reproduction must therefore be so adapted to the times of the tidal and semilunar cycles that the imagines can reach the exposed habitat for egg laying in sufficient numbers.

The intertidal habitat of *Clunio takahashii* from Japan is usually restricted to the mean level, which is exposed and submerged alternately by the semidiurnal cycle

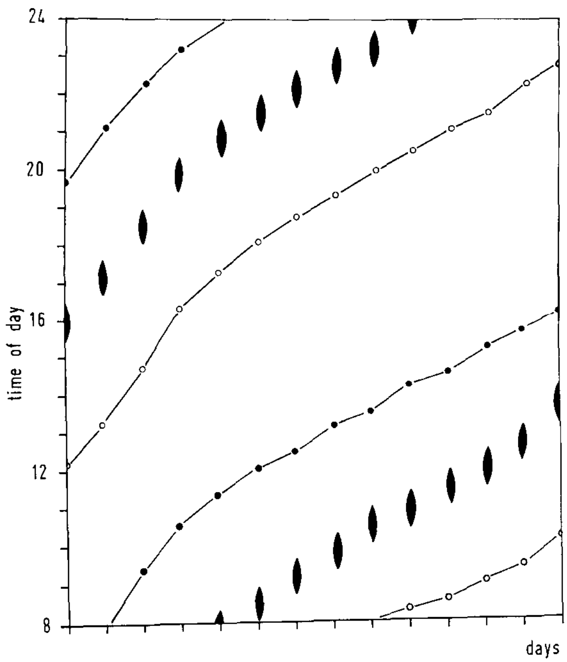

Fig. 1: *Clunio takahashii* – Population in Amami-Oshima/Japan. The changes in emergence time and tides during March 3–16, 1963. ○: high tide, ●: low tide (redrawn from HASHIMOTO 1966)

of tides, through all seasons. In this species HASHIMOTO (1966) observed emergence times, which always occurred twice a day between the times of high and low tide (Fig. 1). The resulting periodicity of emergence is correlated with the semidiurnal cycle of tides. REMMERT (1965) published similar results for a *Clunio* population from the north of Norway. The control of such tidal periodicity of emergence has not yet been analyzed.

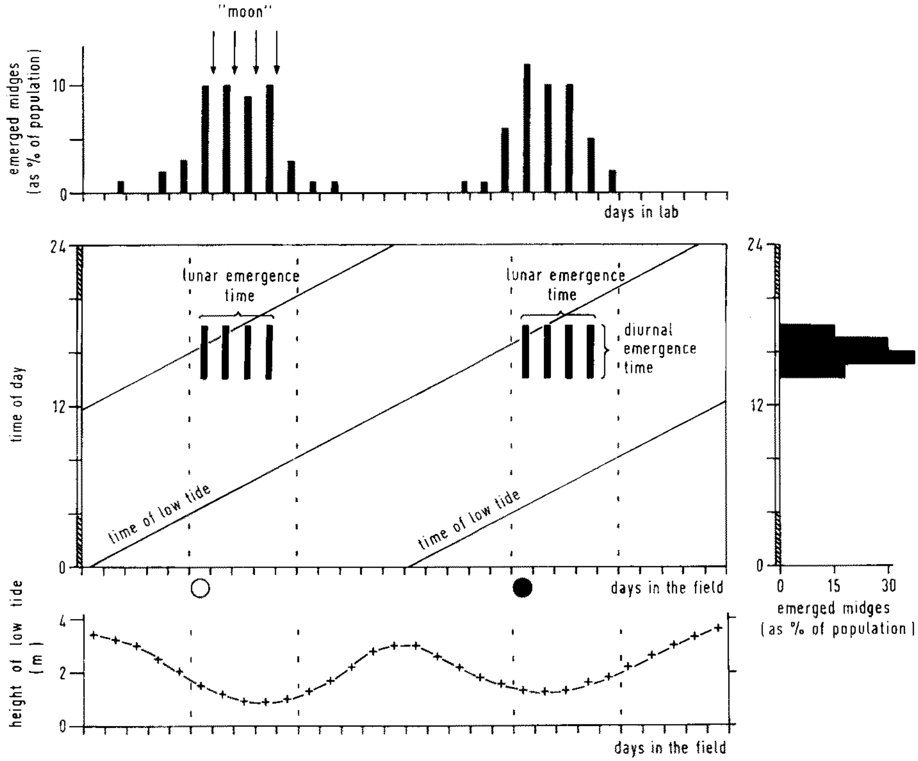

Fig. 2: *Clunio marinus* – Population in Normandy/France. Middle: Diagram of the emergence times in the field (rectangles) and the changes of low tide (diagonals) during one month. Below: Changes in the height of low tide during the same month. Above: Emerged imagos in LD 16:8 with artificial moon-light (every 30 days 4 nights with 0.4 lux). Right: diurnal emergence time in LD 16:8 (light time from 4 to 20 h)

In contrast, the intertidal habitat of *Clunio marinus* from Europe is often located around the lowest level of the intertidal zone, which is only exposed every two weeks about the time of spring low tide. During this time, which lasts only a few days, *Clunio* regularly emerges at about the same time of day (Fig. 2, middle). The resulting periodicity of emergence of this *Clunio* population is therefore characterized by a combination of a semimonthly (semilunar) and a diurnal rhythm. The semimonthly rhythm is correlated with the cycle of spring and neap tides and naturally also with the change in the phases of the moon. The diurnal rhythm corresponds with the time of low tide during the emergence days. The periodicity of emergence can be described only by two dates: the lunar emergence time and the diurnal emergence time (see Fig. 2, middle).

The control of this lunar periodicity has been clarified in the laboratory during the last years (NEUMANN 1966). The semimonthly rhythm is due to a corresponding rhythm of the beginning of pupation. In stocks from Middle and South Europe, it can be evoked by an artificial moonlight cycle (Fig. 2, above). Until now there has been only one interesting exception in *Clunio marinus:* the semilunar rhythm of the North European stock Helgoland was not entrained by the moonlight cycle (detailed discussion in NEUMANN 1966). The diurnal rhythm of emergence can be evoked in all European stocks by an artifical light-dark cycle of 24 hours (Fig. 2, right). Both photoperiodic synchronizers – moonlight cycle and LD cycle – probably control the rhythms also in the field: as far as examined, the phase positions of both rhythms relative to the photoperiodic conditions approximately agree in the laboratory and at the place of origin (Fig. 2).

On coasts with high tidal ranges, the spring tides periodically return 1 to 2 days after full and new moon. However, the semidiurnal cycle of tides may show time displacements of several hours along the coasts (Fig. 3). Thus, the time of spring low tide

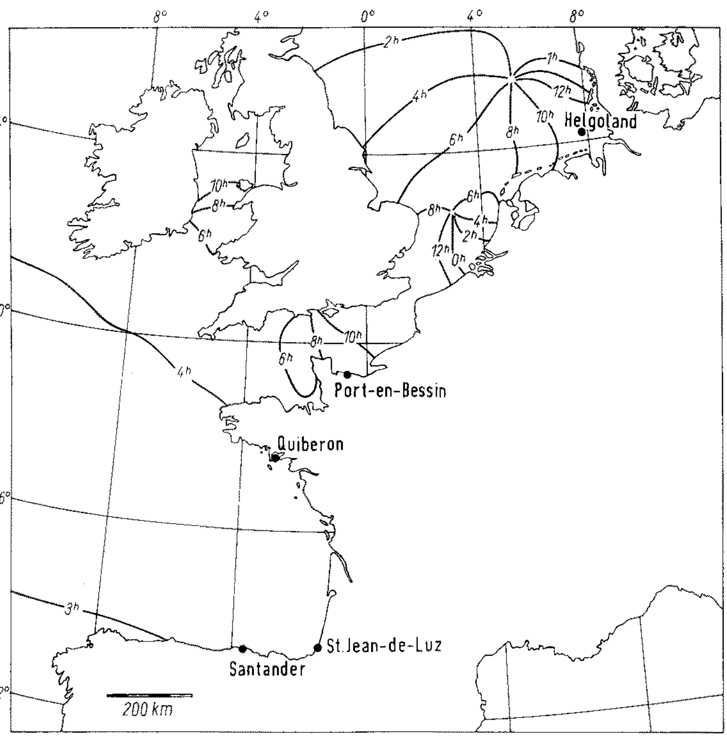

Fig. 3: Locations of the studied populations of *Clunio marinus* and the position of co-tidal lines. Numbers on the lines give the time of high water after the moon's transit of Greenwich (according to data of Deutsches Hydrographisches Institut)

differs between two places of the coast according to the differences in the co-tidal lines. We must expect that populations from different places will differ in their diurnal emergence times in the field and, in consequence of the photoperiodic control of the emergence times, in the laboratory under artificial day–night conditions. This expectation was confirmed by 5 stocks (Fig. 3), which represented isolated populations. By a detailed investigation it was established that the populations of *Clunio marinus* ad-

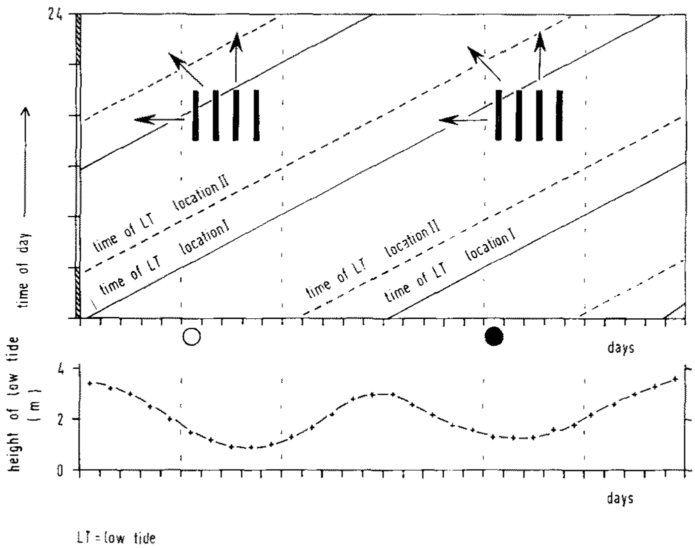

Fig. 4: Possible changes of the lunar and diurnal emergence times (follow the arrows) in the adaptation to different tidal conditions (explanations in the text)

ditionally differ in their lunar emergence times (the emergence days of the synodic month). Let us suppose that a new location with a shifted tidal cycle is settled by a *Clunio* population. Three possibilities exist for the adaptation of the emergence times to the different tidal conditions (Fig. 4): (1) a change in the diurnal emergence time only (vertical arrow), (2) a change in the lunar emergence time (horizontal arrow), (3) a change in the diurnal and in the lunar emergence time (diagonal arrow). If the *Clunio* habitat is located around the lowest intertidal level, which is only exposed during spring tides, then the lunar emergence time ought always to be fixed to the days shortly after full and new moon (as in Fig. 2) and only the diurnal emergence time would be changed according to the differences in the co-tidal lines. This possibility is verified by the populations of Helgoland and Normandy. If the *Clunio* habitat is located between the lowest and the mean level of the intertidal zone, both the diurnal and the lunar emergence time could be changed. This possibility is verified at the places Quiberon, St. Jean-de-Luz and Santander, where the lunar emergence time begins some days before full and new moon, and the diurnal emergence time of each population corresponds to the time of low tide existing at the places of origin during the emergence days.

On coasts with small or no tidal ranges, the emergence times of *Clunio* populations are correlated with the special local conditions. For instance, in the Mediterranean Sea a diurnal cycle of land- and sea-breezes during summer is superimposed on the tidal cycle. Consequently, on the Adriatic Sea (Rovinj/Yugoslavia) a relatively high fall of the tides exists when low tide occurs between midnight and sunrise, during the strongest influence of the landbreeze (Fig. 5). This situation returns every 15 days, paralleling the changes of the phases of moon. Only at these times is the *Clunio* habitat in the zone of the algae *Padina pavonia* and *Halopteris scoparia* exposed, and during these hours the midges are swarming. The semimonthly and diurnal emergence times of the Rovinj population are also controlled by the moonlight and the LD-cycle, respectively, as was shown in the populations from coasts with high tidal ranges.

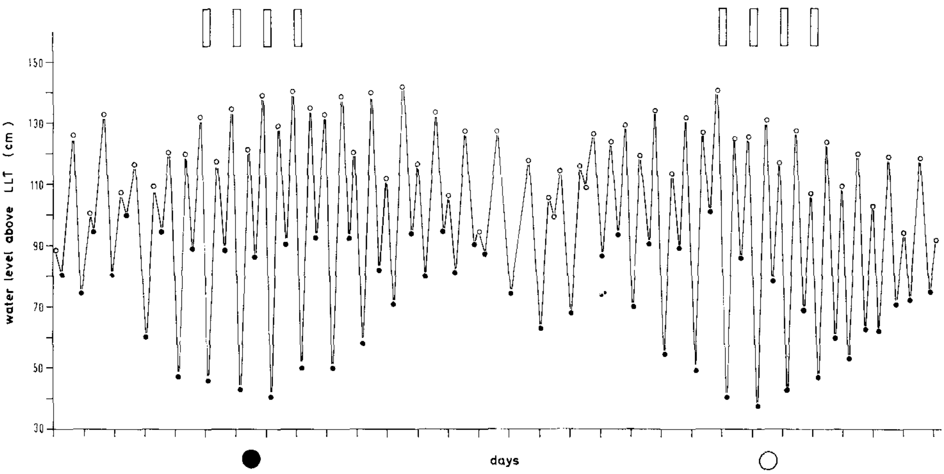

Fig. 5: *Clunio mediterraneus* – Population in Rovinj/Yugoslavia. The calculated emergence time (rectangles) and the tidal range (○: high tide, ●: low tide) during August, 1964

The lunar and the diurnal emergence times are physiological characters which were without doubt selected by the special tidal conditions at the habitat level. In cross-breeding between stocks with different emergence times, one can examine whether these differences are genotypic adaptations. In the case of the diurnal emergence time, such an analysis was possible (Fig. 6). The emergence times of the F_1, the F_2 and the backcross correspond with the inheritance of quantitative characters. A detailed examination of the data showed that the difference in the diurnal emergence time of the two stocks is controlled by only a few genes. One may suppose that such a control by a few genes once might have permitted an advantageous flexibility during the genetic adaptation.

Further investigations of the *Clunio* populations will be interesting for two reasons: (1) *Clunio* offers a favorable example for the analysis of "physiological clocks" because the lunar rhythm of pupation and the diurnal rhythm of emergence are both based on innate rhythms of each individual; the emergence times of the different populations correspond with a different timing of the internal clocks relative to the photoperiodic conditions. (2) *Clunio* represents the rare event that intraspecific differences in physiological characters can exactly be measured and, further, that these differences can accurately be correlated with the regular conditions in the intertidal zone (the

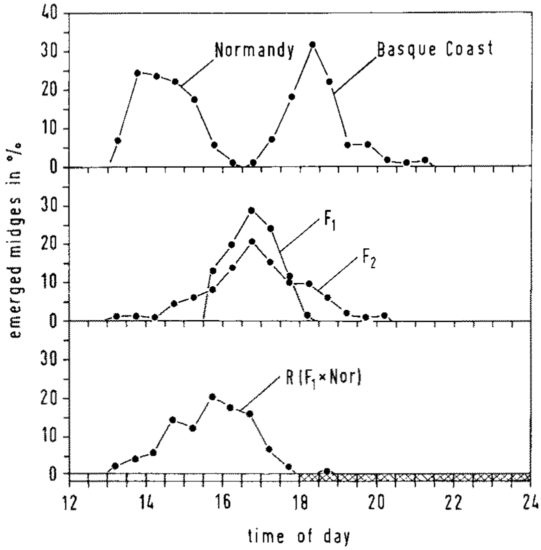

Fig. 6: *Clunio marinus* – Cross-breeding between 2 stocks which differ in their diurnal emergence time. Above: Diurnal emergence time of the parental stocks Normandy (Port-en-Bessin) and Basque Coast (St. Jean-de-Luz) (see Fig. 3), middle: F_1 and F_2-generation, below: Backcross between F_1 and Normandy stock (in all curves only the emergency times of males are projected). Breeding conditions: LD 12:12 light from 6–18 h, 20° C

cycle of tides, the level of the habitat). It may thence be possible to gain insight into the formation of physiological races and the speciation. Such studies are facilitated in *Clunio* since (a) the species has a linear distribution along the coasts and (b) genetic methods are available. It would be advantageous if one could find a test to determine the direction of previous population spreading. Crossing studies have given possible indications for such a test: between the stocks of adjacent populations (Santander–St. Jean, St. Jean–Quiberon) there existed a unilateral crossing ability. This non-reciprocal crossability may represent a specialized mechanism, which was effective during the formation of the physiological races and which allowed a reproductive isolation in the direction of spreading and a gene flow in the opposite direction (NEUMANN, in preparation for press).

SUMMARY

1. The emergence times of intertidal *Clunio*-species (Diptera, Chironomidae) are correlated with special tidal conditions in such a way that the immediately following reproduction of the short-lived imagos can take place on the exposed habitat.

2. If the habitat of a *Clunio*-species is situated in the middle tidal region and exposed twice a day by the tidal cycle (T = 12.4 h), a tidal rhythm of emergence with an average period of 12.4 hours may result (example: *Clunio takahashii*).
3. If the habitat is located in the lower tidal zone, exposed only at about the time of the spring tides, a semilunar rhythm of emergence is expected (examples: *Clunio marinus* and *Clunio mediterraneus*). These semilunar rhythms are correlated with certain conditions of low tide which occur at the coastal locations every 15 days at about the same time of day. The semilunar rhythm is therefore exactly characterized by two dates: the lunar emergence time (a few successive days around full and new moon) and the diurnal emergence time.
4. According to experimental investigations on the control of the emergence rhythm, the midges are able to determine both dates in advance.
5. Coastal populations differ in their lunar and diurnal emergence times. These differences correspond to the time of low tide which exists at each location during the emergence days of the semilunar rhythm.
6. Crossbreeding between stocks of different populations showed that the differences in diurnal emergence time are gene-controlled.

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Discussion following the paper by NEUMANN

CRISP: The closely defined and genetically controlled mechanism of emergence suggests that it must have great adaptive value. Yet from time to time low water may be delayed by winds or prevented by low barometric pressures. On such occasions is there mass mortality, or does *Clunio* have a double assurance mechanism to prevent emergence on such occasions?

NEUMANN: I do not know of any indication for such mechanism. According to oberservations in the field, the diurnal emergence time was not shifted by an abnormal tidal level. This was confirmed in the laboratory by experiments with a tide generating machine (unpublished data). On such abnormal occasions, most of the imagines cannot reach the appropriate habitat for egg laying. Hence there will probably occur a mass mortality among embryos and first instars.