

TEGULA FUNCTION DURING FREE LOCUST FLIGHT IN RELATION TO MOTOR PATTERN, FLIGHT SPEED AND AERODYNAMIC OUTPUT

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Summary

Tegulae are complex proprioceptors at the wing base of locusts. Deafferentation of the tegulae causes a lack of specific phasic information related to the wing downstroke and the timing of the upstroke. Employing telemetry during free flight of the locust *Schistocerca gregaria*, we investigated the consequences of tegula ablation on free flight parameters including motor patterns (wingbeat frequency and the relationship between the activation of flight muscle antagonists), free flight speed and aerodynamic output. We investigated the role of the tegula pairs of both wings on the motor pattern generated in free-flying locusts. We show that the tegula organs are not essential for generating the motor patterns necessary for

free flight. However, they are required for increasing the motor output to give additional effective lifting power during adaptive behaviour. We also investigated long-term changes in the free flight parameters after tegula ablation. The recovery of the adult flight system revealed in the present study suggests that there is adaptation to the loss of proprioceptive information; this argues for a full functional and behavioural recovery of the flight system of the locust under closed-loop conditions.

Key words: tegula, telemetry, locust, *Schistocerca gregaria*, free flight, motor pattern, aerodynamic output.

Introduction

During locust flight, the modulation of motor patterns underlying flight behaviour is mediated by a set of sense organs (Wendler, 1974). In particular, the wing hinge stretch receptors (Kutsch, 1974; Pearson et al., 1983), the antennae (Gewecke, 1975), the system of frontal wind-sensitive hairs (Gewecke, 1975; Horsmann et al., 1983) and the tegula organs (e.g. Kien and Altman, 1979; Büschges and Pearson, 1991) have been studied. Afferent information from such proprioceptive sensory organs participates in flight pattern generation: studies of tethered flying animals have demonstrated that information from the wing-associated sense organs modulates muscle activity patterns (Neumann et al., 1983; Möhl, 1985; Wolf and Pearson, 1988).

The tegulae are complex sense organs on the anterior base of each wing; they consist of a knob-shaped hair field (the cupula) and internal chordotonal sensilla (Kutsch et al., 1980). The tegulae are excited in parallel with the downstroke movement of the wings (Neumann et al., 1983). Tegula afferents project onto identified interneurons related to the central pattern generator (Pearson and Wolf, 1988). Phasic input from the tegulae was found to be partly responsible for the initiation of elevator activity and, therefore, for the timing of the elevator muscles within the wingbeat cycle (Wolf and Pearson, 1988). The tegula organs of the fore- and hindwings are not equally important in generating flight patterns: partial deafferentation experiments

have shown that the forewing organs are of minor, if any, importance for the neural generation of basic elevator–depressor flight patterns (Büschges and Pearson, 1991). The flight system can recover from the loss of wing proprioceptive sense organs (Kutsch, 1974; Büschges et al., 1992a,b; Gee and Robertson, 1996), suggesting synaptic plasticity within the nervous system (Wolf and Büschges, 1997).

Although detailed information is available about the interaction between tegula afferent input and the centrally generated flight pattern, there are few reports concerning tegula function. In experiments involving tethered flying locusts, Wolf (1993) pointed out that tegula input may be involved in the control wing movement and in wing-related aerodynamic force production. Investigating the characteristics of free flight in locusts recovering from tegula ablation, Gee and Robertson (1998) reported an overall deficit in free flight ability following 3–4 weeks of recovery from hindwing tegula ablation and impairment of free flight ability in locusts recovering from forewing tegula removal. These observations are inconsistent with previous studies of tethered flight, which have demonstrated complete recovery of the flight motor pattern following ablation of the hindwing tegulae (Büschges and Pearson, 1991) and no significant effects of the removal of the forewing tegulae on the flight motor pattern (Büschges et al., 1992a,b).

In seeking an explanation for these different findings, it is necessary to examine the behavioural consequences of the changed motor output that follows sensory deafferentation during locust free flight. Among the questions to be answered are the following. (a) Does the decrease in the wingbeat frequency after tegula removal (e.g. Büschges and Pearson, 1991) cause a lowering of motor output to levels inadequate for maintaining the general free-flight capability? (b) Is the phasic information mediated by the tegula involved in, or even essential for, the regulation of behavioural parameters such as the free flight speed? (c) Does the recovery of the central flight patterns following tegula ablation accompany a recovery of the behavioural parameters such as speed and lift generation during free flight?

In the present study, therefore, we focus on changes in the behavioural parameters of free-flying locusts following tegula ablation. Telemetric acquisition of electromyographs (e.g. Kutsch et al., 1993) by a modified low-weight device enables analysis of motor patterns during free flight of intact and sensory-ablated locusts. This approach is used for a simultaneous investigation of the motor output of the locust flight system and related behavioural parameters, such as speed and aerodynamic force production during free flight. We determined the motor and behavioural output during free flight and evaluated the significance of the tegula organs in generating the motor pattern underlying the capability for free flight. In addition, we investigated the recovery of free flight behaviour following removal of either the forewing or the hindwing tegulae and after ablation of all tegula organs.

Materials and methods

Animals and flight conditions

All tests were carried out using female locusts *Schistocerca gregaria* Forskål. The animals were bred at 42°C and 70% relative humidity in crowded populations of both sexes with a 12h:12h light:dark cycle. Maturation occurred 18 days after final ecdysis (determined by observations of copulating pairs). Individual flight sequences were investigated in a room measuring 10 m×5.5 m×2.2 m. The room temperature could be adjusted within the range 18–40°C. Unless stated otherwise, the experiments were performed at 34°C. The first flight activity of each individual was induced by horizontal acceleration ($v_0 \approx 2.0 \text{ m s}^{-1}$, where v_0 is the initial flight velocity), which was immediately followed by spontaneous flight sequences by the animal. Flight duration varied between 5 and 30 s per flight sequence. In each type of experiment, each individual was restricted to three induced and five spontaneous flight sequences (summed flight time per individual averaged 1.5 min). Wherever possible, spontaneous flight sequences have been evaluated.

Telemetric device for recording electromyographs during free flight of locusts

For telemetric acquisition of electromyographs (EMGs), the

one-channel transmitter design of Kutsch et al. (1993) has been modified: the transmitter mass has been reduced to 0.3 g including the power supply using 0605 and 0805 SMD components (Bürklin, Germany) and a lithium battery (2.2 V, 3.3 mA; Renata, Switzerland). The transmitter design and its attachment to the pronotum are shown in Fig. 1A,B. The transmitter-specific VHF carriers were adjusted in the range from 128 to 145 MHz. An HB9CV aerial (32 dB amplification) was used for signal detection. A wide-band receiver (consisting of Harris modules, UK) was used to demodulate the signals.

Electromyograph wires (Manganin, Isabellenhuetten Dillenburg, Germany; 50 µm in diameter, insulated except at the tip) were inserted into one of the hindwing depressor (D) muscles, M129, and one of the hindwing elevator (E) muscles, M113 (nomenclature after Snodgrass, 1929), both electrodes being connected to the preamplifier input. A further electrode wire was inserted in the abdominal haemocoel as a reference. Typical telemetric EMGs from these antagonistic muscles during free flight are shown in Fig. 1C.

Tegula ablation and post-experimental control

To remove the hindwing tegulae, the animals were taped ventral side down onto a cork platform with the forewings unfolded. The exposed hindwing tegulae were ablated using forceps with sharpened spoon-shaped tips specially designed for tegula ablations (see also Wolf, 1993). Ablation of the forewing tegula organs required turning the animal sideways and enlarging the junctions between the pro- and mesothorax. Manipulations were carried out under a dissecting microscope to avoid damage to the wing base or other related structures. Animals from control groups were treated identically except that the tegula organs were not removed (sham-operation). Removal of the cupula in the fore- and hindwing cut NIC1a, but NIC1b remained intact in all the experimental animals (for nerve nomenclature, see Campbell, 1961; Kien and Altman, 1979).

In addition, the successful removal of the forewing tegulae was checked after the free-flight experiments by recording extracellularly from nerve NIC1 (Fig. 2). The animals were fixed to a holder by their ventral surface, and the episternal cuticle was opened by cutting a narrow window immediately below the first basalar sclerite. Recordings from NIC1 were made proximal to branches NIC1a and NIC1b using a silver hook electrode (30 µm in diameter). Stimulation of the nerve was carried out by passive movement of the forewing. In the case of the hindwing tegulae, *post mortem* checks were carried out to confirm that NIC1a had been cut and that NIC1b was intact. We did not record from the metathoracic NIC1 since it was not possible to evoke adequate stimulation by moving the complete hindwing, which has a soft morphology.

Recording technique and analysis of free flight in locusts

Free-flight sequences of individual locusts were recorded using a video camera (Panasonic NV-MS1EG; frame

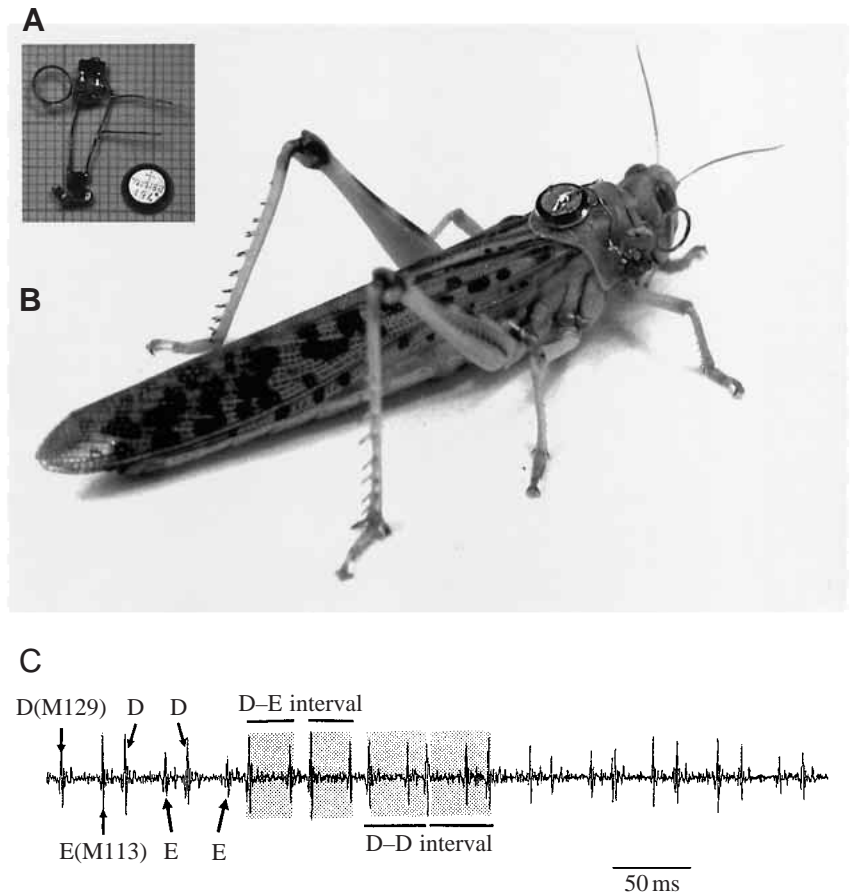


Fig. 1. (A) Advanced telemetric device for recording electromyograms (EMGs) of locusts during free flight (mass 0.3 g, battery included) using SMD components (scale: small squares 1 mm^2). For basic circuitry and a detailed description of the prototype, see Kutsch et al. (1993). (B) An adult female *Schistocerca gregaria* with the transmitter device attached to the pronotum using beeswax resin. (C) Telemetric EMG of two hindwing antagonists (depressor M129, D, and elevator M113, E) during free flight (at 34°C). Intervals D–D and D–E are marked by horizontal bars. The phase relationship ϕ (i.e. the phase of the elevator within the depressor cycle) is calculated as $\phi = (D-E)/(D-D)$.

frequency 50 Hz, shuttering time 1 ms, frame interval 20 ms). The EMGs were displayed on an oscilloscope (Tektronics D11) and stored on tape (Racal Store 4DS). The oscilloscope screen was filmed using a second video camera (Revue Video 8; frame frequency 50 Hz, shuttering time 1 ms), and the synchronous video outputs of both cameras were mixed on-line (Panasonic Digital Mixer WJ-AVE5) and recorded on a video master tape.

Free flight parameters

Before the experiments, the mass and body length of each individual were determined. Free flight parameters were acquired by frame-by-frame analysis of the continuous displacement of the body silhouettes stored on the video master tape (Fischer, 1998). The centre of gravity (at approximately 50% of body length; Weis-Fogh, 1956b; Fischer, 1994) was chosen as a reference point on the body. The parameters obtained were, if necessary, transformed with reference to the parallax of the video recordings (see also Baker and Cooter, 1979). All parameters are considered as 'instantaneous' flight parameters since they were constant within the frame interval of 20 ms.

Parameters describing the motor patterns during free flight

Instantaneous D–D (i.e. cycle length), D–E and E–D intervals (see Fig. 1C) (all measured in ms) were determined

from simultaneous telemetric EMGs of M129 (D) and M113 (E). Instantaneous wingbeat frequencies (Hz) were deduced from the D–D intervals. The instantaneous elevator phase (ϕ) within the depressor cycle was calculated from $\phi = (D-E)/(D-D)$ and describes the relationship between the activation of the two antagonists during a wingbeat cycle.

Parameters monitoring behaviour during free flight

The following behavioural parameters were investigated during free translatory movement of an animal: (a) ascent angle α to the horizontal (degrees) (i.e. vertical flight path); and (b) flight speed v (m s^{-1}). Here, flight capability is defined as the ability to maintain at least continuous level flight (i.e. $\alpha \geq 0^\circ$ within the evaluated flight sequences), indicating that the animal is able to generate sufficient lifting power to support its body mass.

Parameters monitoring the aerodynamic output of the flight system during free flight

Unlike the tethered flight approach, which allows direct 'weighing' of vertical and horizontal force components (e.g. Weis-Fogh, 1956b; Kutsch and Gewecke, 1979; Wolf, 1993), it is not possible to acquire measurements of aerodynamic forces during free flight. During free flight, the aerodynamic output of the flight system is dependent on the ascent angle α

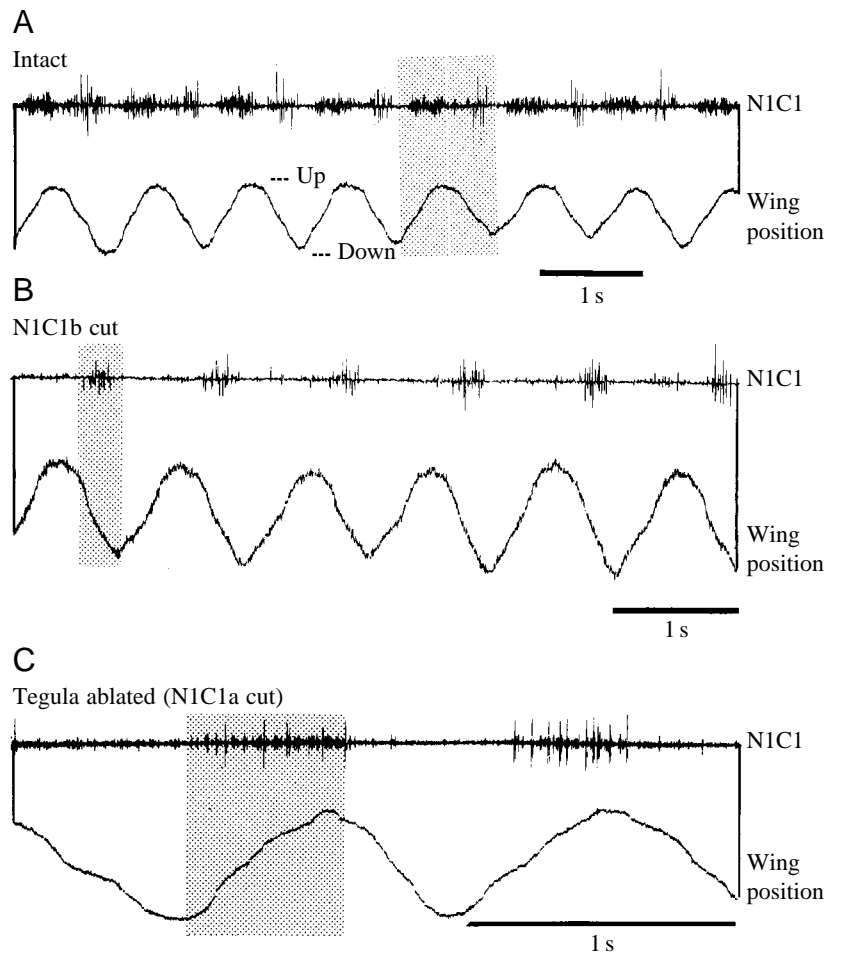


Fig. 2. Extracellular recordings of the mesothoracic NIC1 activated by passively moving the forewing in ventrally fixed animals. The traces show activities recorded in NIC1 with C1a and C1b intact (A), and with C1b cut (B) (traces in A and B are from the same individual). (C) Typical activity recorded in NIC1 after removal of the tegula organ (C1a cut, C1b intact). For all traces, the phasic activities are marked in relation to forewing movement (lower traces in A–C) by shaded areas. Deafferentation of the tegulae causes a lack of specific phasic information in NIC1 afferents from the downstroke; however, NIC1 phasic afferents related to the wing upstroke (probably mediated by campaniform sensilla; e.g. Fig. 1A in Kien and Altman, 1979) are still present. Wing movement was performed by a servomotor device; wing position was recorded by a potentiometer.

and can be monitored as the instantaneous effective lifting power P_{EL} (W), calculated as:

$$P_{EL} = m^* g v \sin \alpha,$$

where m^* is the body mass of the animal in kg, transmitter mass included, g is the gravitational constant ($g = 9.81 \text{ m s}^{-2}$), v is the instantaneous flight speed (m s^{-1}) and α is the instantaneous ascent angle to the horizontal (degrees). P_{EL} is the instantaneous vertical power component additionally generated by the animal when changing from level flight [$\alpha = 0^\circ$; $P_{EL}(0^\circ) = 0 \text{ W}$] to ascending flight at a given value of α .

Fixing the transmitter device to the pronotum increased the original body mass m of the animals (ranging from 2.8 to 3.3 g for the adult females used here) by 8–12%. Adult females of *S. gregaria* are able to carry approximately 30% additional body mass without losing the capability for horizontal flight (indicating the ability to support their body weight during flight, see Fig. 1A in Fischer et al., 1997). There was no significant difference between the free flight parameters (speed, body angle, ascent angle, P_{ELmax} and f_{WB} , where f_{WB} is wingbeat frequency) for freely flying adult female *S. gregaria* either unloaded or loaded with a 0.3 g transmitter (for details, see Fischer, 1998, p. 70). Although the body mass m^*

(which, on average, increased by less than 10% compared with m) also contributes to P_{EL} , values of P_{EL} are mainly dependent on the observed flight speed (v) and the ascent angle (α).

Statistics

Independent samples of the animal populations were obtained by replacing the animals into the specific stem population after the measurements. In each specific set of measurements, the data from all animals were pooled. All sets of data were tested for normal distribution (χ^2 ; $P < 0.01$) and for homogeneity (by analysis of variance, ANOVA; $P < 0.05$). Comparisons of two homogeneous sets of data were performed using Student's t -test; where inhomogeneity occurred, the m -test of Dixon and Massey (1969) was used. For non-parametric comparisons, the Mann–Whitney U -test was applied. Comparisons of multiple data sets were made using the Kruskal–Wallis H -test. Regression analysis was performed using the least-squares difference method (LSD; e.g. Sachs, 1978). The significance of linear and non-linear regressions was tested using the coefficient of determination r^2 .

Mean phase values ϕ of the elevator activity within the depressor cycle were calculated using circular statistics (see Batschelet, 1981). Mean phase values are given as $\phi \pm$ mean angular deviation, with r describing the mean vector. Circular

two-sample comparison was performed using the Watson–Williams test. Results are presented as means \pm s.d. (N).

Results

Free flight motor pattern after partial and complete tegula ablation

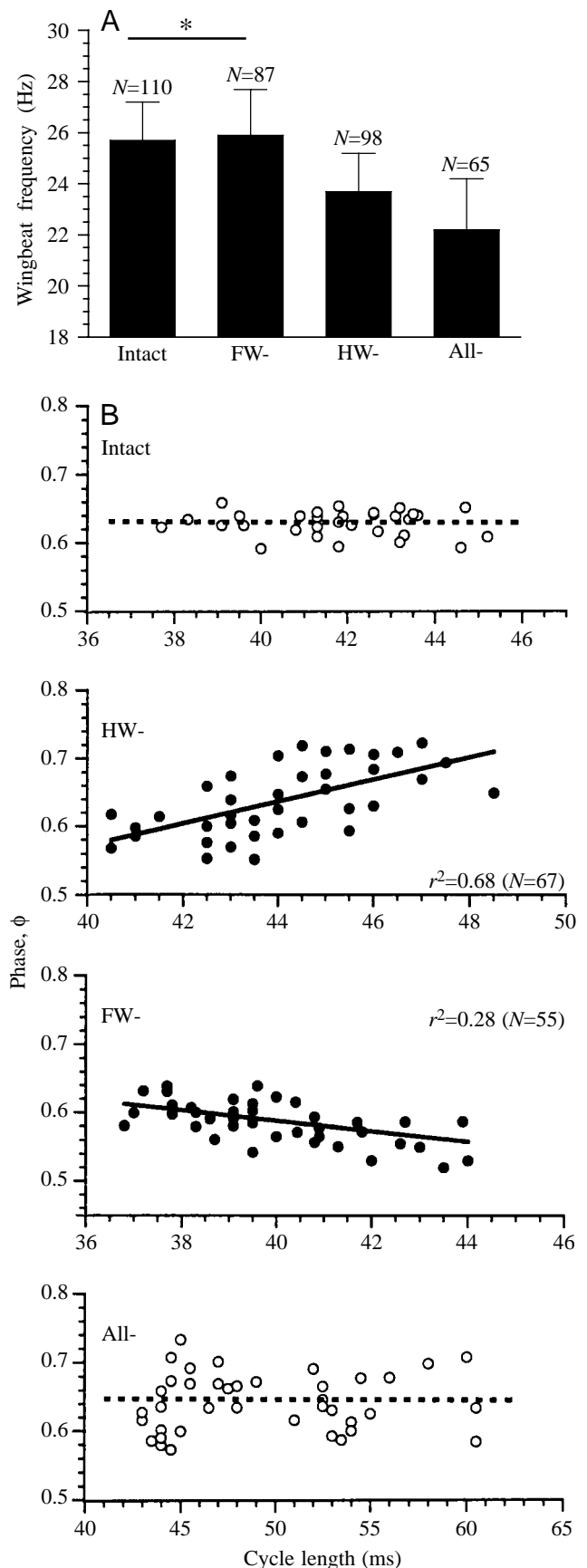
Effects on wingbeat frequency

The instantaneous wingbeat frequencies (f_{WB}) at 34 °C of 12 animals were determined by analyzing the telemetric EMGs. The mean f_{WB} after ablation of the specific tegula organs are given in Fig. 3A. After removal of the hindwing tegulae, f_{WB} decreased significantly from 25.7 ± 1.5 Hz (mean \pm s.d.; $N=110$) to 23.7 ± 1.5 Hz ($N=98$). The removal of all tegulae caused a further decrease in f_{WB} to 22.2 ± 2.0 Hz ($N=65$). After removal of the forewing tegulae, f_{WB} did not differ significantly from the control value (25.9 ± 1.8 Hz, $N=87$). The capability for free flight was maintained for all these animals.

Effects on phase relationships of muscle antagonists

The phase (ϕ) relationships during free flight after partial and complete tegula ablation were determined by analysing the telemetric EMGs of the metathoracic antagonists M129 (D) and M113 (E) (Fig. 3B). Ablation of the hindwing tegulae caused a significant increase in the mean D–E interval from 24.2 ± 2.5 ms ($N=63$) to 28.5 ± 3.0 ms ($N=45$, $P < 0.05$). The D–E interval increased significantly to 29.6 ± 4.0 ms ($P < 0.05$) following removal of all tegulae. No significant change in mean D–E interval was observed after ablation of the forewing tegulae. In controls, there was no significant dependence of the instantaneous elevator phase on the actual cycle length (i.e. D–D) ($P > 0.05$). After ablation of the hindwing tegulae, ϕ became positively correlated with cycle length ($P < 0.05$). This phase shift corresponds to an increase in the D–E interval and indicates a delay in elevator activation during the wingbeat cycle when cycle length is increased. After removal of the

Fig. 3. (A) Instantaneous effects of tegula ablation (FW-, forewing tegulae removed; HW-, hindwing tegulae removed; All-, fore- and hindwing tegulae removed) on the wingbeat frequency f_{WB} during free locust flight ($T=34$ °C). Values are presented as means \pm s.d. An asterisk marks values that are not significantly different ($P > 0.05$). The flight capability of all individuals was maintained even after complete tegula ablation. (B) Phase (ϕ) relationships of the metathoracic antagonists M129 (D) and M113 (E) during free flight following partial and complete ablation of the tegula organs. Data from 12 individuals (at 34 °C). Dashed lines indicate that the mean value of ϕ is independent of the instantaneous cycle length D–D. After removal of the hindwing tegulae, ϕ became dependent on the cycle length D–D (i.e. on f_{WB}), indicating a delayed upstroke when cycle length D–D is increased. After deafferentation of the forewing tegulae, there was also a significant, but smaller, shift in ϕ negatively correlated to the cycle length (i.e. earlier elevator activation when D–D is increased). Note the general increase in D–D (see ordinate scaling) after hindwing tegula removal. However, there was no significant increase in D–D after forewing tegula ablation.



remaining forewing tegulae, the elevator phase ϕ was found not to be significantly correlated with the actual cycle length ($P>0.05$). The flight muscle pattern after ablation of the forewing tegulae showed a slight but significant negative correlation between ϕ and cycle length ($P<0.01$), which corresponds to a reduction in the D–E interval and indicates an earlier activation of the elevator when cycle length is increased.

No significant difference ($P>0.05$) in the mean phase relationship of the antagonists was found between controls ($\phi=0.629\pm 0.018$, $r=0.994$; $N=75$) and after ablation of all tegula organs ($\phi=0.636\pm 0.0394$, $r=0.968$; $N=45$).

Instantaneous effects of tegula ablation on free flight behaviour

Free flight speed

Instantaneous flight speeds of two populations (each with 12 individuals) were determined after specific tegula ablation (Fig. 4). After control flights (sensory input intact) of both populations, the hindwing tegulae of one population were ablated while the forewing tegulae of the other group were ablated. After flight speed had been measured again, the remaining tegulae were removed in both populations to give complete ablation.

The mean flight speed of the intact animals ($4.1\pm 0.5\text{ m s}^{-1}$, $N=80$) did not change significantly ($P>0.05$) after removal of the forewing tegulae ($4.0\pm 0.6\text{ m s}^{-1}$; $N=77$), but subsequent removal of the hindwing tegulae resulted in a significant decrease in the mean flight speed to $3.2\pm 0.4\text{ m s}^{-1}$ ($N=101$, $P<0.05$). A slightly but significantly reduced flight speed ($4.2\pm 0.6\text{ m s}^{-1}$, $N=50$; intact mean $4.5\pm 0.6\text{ m s}^{-1}$, $N=64$) was observed when only the hindwing tegulae were removed

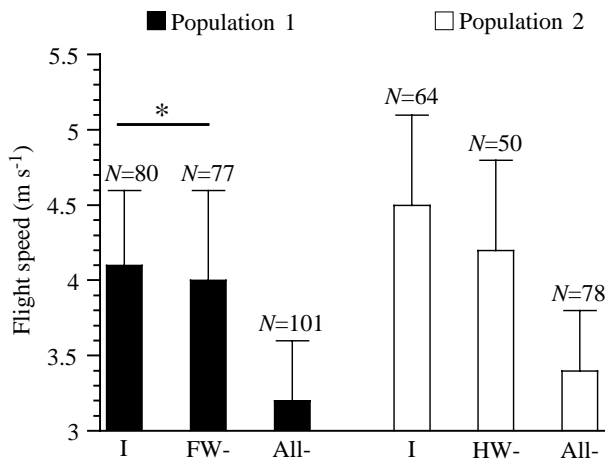


Fig. 4. Instantaneous effects of tegula removal on the mean flight speed during free locust flight (at 34 °C) investigated in two populations with a different test program (each with 12 adult females aged 18 days). An asterisk indicates that no significant decrease in flight speed was observed ($P>0.05$) following ablation of the forewing tegulae (FW-). Removal of all tegulae (All-) caused a significant decrease in flight speed in both populations. The flight capability of all the animals was maintained even after ablation of all tegulae. Values are means + S.D. I, intact animals.

($P<0.05$). In this population, removal of the remaining forewing tegulae caused a significant decrease in the mean flight speed ($3.4\pm 0.4\text{ m s}^{-1}$, $N=78$). Both populations showed comparable mean flight speeds after deafferentation of all tegulae (means not significantly different, $P>0.05$). Flight capability was maintained in all these individuals.

Aerodynamic output

The non-linear characteristics of P_{EL} as a function of α (r^2 significantly different from zero, $P<0.01$) for the populations investigated are shown in Fig. 5. Maximum effective lifting power P_{ELmax} at $\alpha=\alpha_{max}$ was calculated from the equations used to fit the data. The values obtained for P_{ELmax} after ablation of the forewing tegulae (54.8 mW, population 1) or the hindwing organs (49.9 mW, population 2) did not differ significantly from the control values (50.4 mW for population 1; 49.8 mW for population 2). After ablation of all tegula organs, a similar decrease in P_{ELmax} was seen in both populations (to 32.2 mW for population 1; to 30.9 mW for population 2). These findings indicate that even after the removal of all tegula organs (a) the aerodynamic output of the flight system is sufficient to maintain flight capability and to enable ascending flight; and (b) the observed range of ascent angles (i.e. the vertical flight paths) is limited to lower values than those for controls.

Long-term changes in free flight parameters after tegula ablation

Flight speed

Adult individuals ($N=30$) from one colony were investigated. Three days after final ecdysis, the hindwing tegula organs were removed from 10 animals, and all tegulae were ablated in a further 10 individuals. Ten animals were left intact to serve as controls. Sampling was first carried out 1 day after ablation; then, starting 9 days after ablation, consecutive measurements were performed at 3 day intervals until 18 days after ablation. The mean flight speeds of the intact and deafferented groups are shown in Fig. 6. During the period of observation, the speed of the sensory-intact animals increased significantly from $3.2\pm 0.6\text{ m s}^{-1}$ to $5.1\pm 0.6\text{ m s}^{-1}$ (mean \pm S.D.; $P<0.05$). For individuals without hindwing tegulae, flight speeds over the first 15 days were significantly lower than those of the sensory-intact group ($P<0.05$); however, no significant difference was found on the eighteenth day of observation. The flight speed following hindwing tegula ablation increased significantly from $2.7\pm 0.4\text{ m s}^{-1}$ on day 1 to $4.9\pm 0.7\text{ m s}^{-1}$ ($P<0.05$) on day 18. No significant increase in flight speed was observed over 18 days ($P>0.05$) in individuals without any tegulae, and by the end of the observation period the flight speed was only 58 % of that of sensory-intact locusts. All individuals maintained their capability for free flight.

Aerodynamic output

Long-term changes in P_{ELmax} have been investigated (data not shown) and, depending on maturation, a general increase in this output was found in intact animals with values of P_{ELmax}

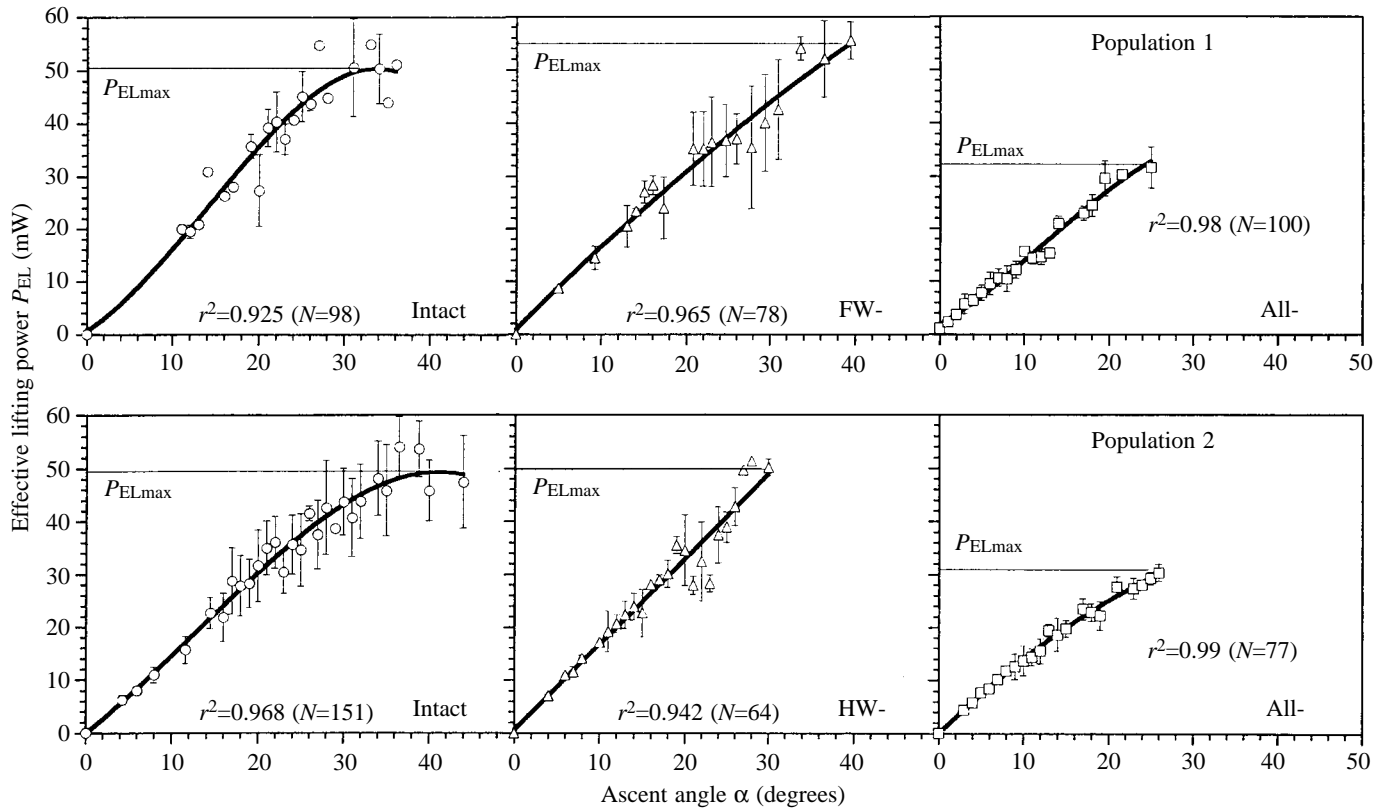


Fig. 5. Effective lifting power P_{EL} in relation to the ascent angle α observed in intact and tegula-ablated animals (two populations; see Fig. 4). When different values were obtained for a given α , the mean value \pm s.d. is plotted. Maximum effective lifting power P_{ELmax} was either calculated from the characteristics of the relationship between α and P_{EL} or from the maximum observed P_{EL} at $\alpha=\alpha_{max}$. All r^2 values shown are significant ($P<0.01$).

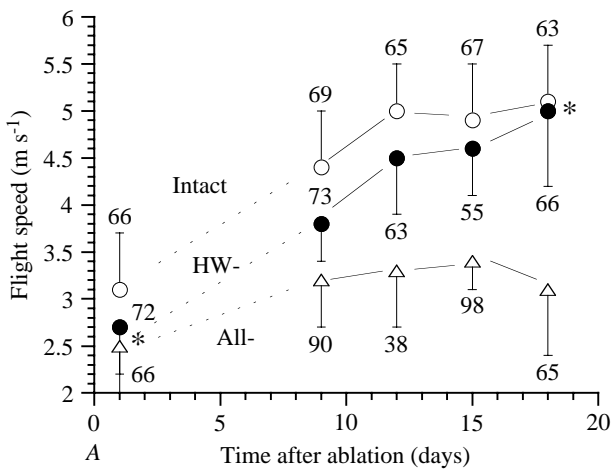


Fig. 6. Changes in the free flight speed (mean \pm s.d.) in intact animals (open circles), after ablation (A) of the hindwing organs (filled circles) or after removal of all tegulae (open triangles; each group contained 10 individuals). Deafferentation was carried out 3 days after final ecdysis, and first sampling was at 1 day after ablation (i.e. at 4 days of adult age). Each mean consists of N samples of all 10 individuals within each group, with the value of N given beside the data points. An asterisk indicates that means do not differ significantly ($P>0.05$). For details, see text.

increasing from 57 mW on day 9 to 78 mW at 18 days. For individuals with all tegulae removed, no significant changes in the output were observed (P_{ELmax} at 9 days, 41 mW; at 18 days, 46 mW, which is approximately 60% of the intact value). After removal of the hindwing tegula organs, P_{ELmax} was 50 mW on the ninth day of observation and this increased to 71 mW on day 18. Both values did not differ significantly from the values found in the control population.

Wingbeat frequency

The experiments described here were carried out on a population of 80 females from one colony. This colony was initially divided into four groups, each containing 20 animals. Three days after final ecdysis, 20 individuals were deafferented by removal of either the fore- or the hindwing tegulae; in a further 20 individuals, all tegulae were ablated. Twenty animals served as controls. Five animals in each group were first tested 9 days after ablation, and five other individuals from each of these groups were tested every third day. The mean values of f_{WB} were determined from telemetric EMGs of M129. In all groups, f_{WB} was observed to change with maturation: f_{WB} increased significantly from 23.9 ± 1.6 Hz ($N=66$, 9 days) to 25.9 ± 1.8 Hz ($N=88$, 18 days, $P<0.05$) in controls, and from 20.8 ± 1.9 Hz ($N=81$) to 24.5 ± 1.4 Hz ($N=67$; values mean \pm s.e.m., $P<0.05$) in animals with all tegulae

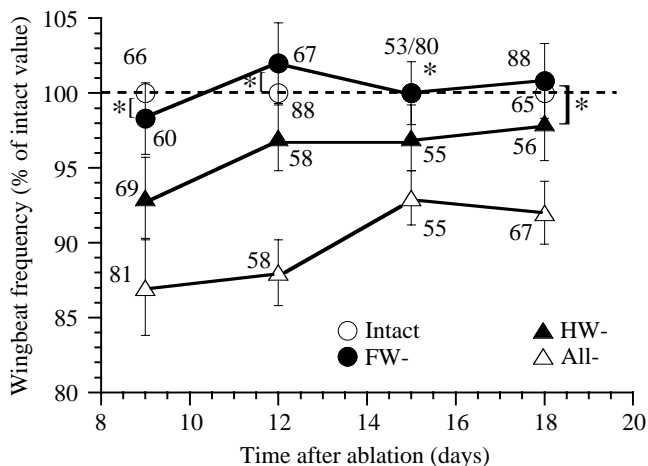


Fig. 7. Changes in wingbeat frequency (mean \pm S.D., calculated from telemetric electromyograms of M129) in intact animals and in animals with partially ablated (HW- or FW-) tegulae or completely removed (All-) tegula organs. Ablation was performed 3 days after final ecdysis, and the first measurements were made 9 days after ablation. The values are given as a percentage of the mean value observed in intact animals. Each mean was obtained from N observations from five animals per group, with the value of N given beside the data points. An asterisk indicates that means do not differ significantly ($P > 0.05$). For details, see text.

ablated. These changes in f_{WB} (as a percentage of that of the intact group) are plotted in Fig. 7. Over the period of observation, the mean f_{WB} of individuals deafferented by forewing tegula removal was not significantly different from that of the controls ($P > 0.05$). For the group with the hindwing tegulae removed, the mean f_{WB} was significantly lower than that of controls over the first 15 days, but no significant difference was found on the eighteenth day of observation ($P > 0.05$). The mean f_{WB} of individuals after removal of all tegulae was always significantly different (between 88% and 93%) from the value found in controls. Free flight capability was retained by these locusts throughout the period of observation.

'Minimum' wingbeat frequency required for free flight

To evaluate the role of the tegula organs in the generation of the motor pattern that underlies free flight (i.e. the motor output adequate to support the body weight), we first determined the 'minimum' f_{WB} needed for free horizontal flight ($\alpha = 0^\circ$).

During continuous level flight ($\alpha = 0^\circ$), f_{WB} was quantified by telemetry of M129 activity at three different environmental temperatures (18, 27 and 34 °C). In a population of nine intact individuals, no ascents where $\alpha \geq 0^\circ$ were observed at a temperature lower than 18 °C. In most animals, no flight activity was documented at all below 18 °C. Continuous level flight ($\alpha = 0^\circ$) was first observed at 18 °C (but only in three of the nine animals). These animals exhibited a mean f_{WB} of 17.3 ± 1.9 Hz ($N = 45$). For animals with a lower f_{WB} , only descents were observed at 18 °C. We conclude, therefore, that

the minimum f_{WB} (i.e. the minimum motor output) required for free flight in adult female *S. gregaria* is approximately 17 Hz.

At 27 °C, six of the nine animals showed level flight, and the mean f_{WB} was determined to be 23.3 ± 1.3 Hz ($N = 78$). All nine animals were capable of free flight at 34 °C, with a mean f_{WB} of 25.6 ± 1.5 Hz ($N = 103$). The mean f_{WB} after removal of all tegula organs was 22.9 ± 1.8 Hz ($N = 77$) at 34 °C. All the animals retained their capability for free flight after removal of all tegulae.

We conclude from these observations that, even after the loss of all afferent information from the tegulae, the capability for free flight (at 34 °C) is maintained because the wingbeat frequency is still approximately 5 Hz above the minimum value generally required to support the body weight.

Discussion

Significance of the tegulae for motor pattern generation during free flight

The tegula organs are wing proprioceptors that are considered to be necessary for the generation of a functional flight motor pattern (Wolf, 1993). It has been shown in tethered flying preparations that ablating the hindwing tegulae increases the time interval between activation of the wing depressor and elevator muscles, consequently decreasing the wingbeat frequency (Wolf and Pearson, 1988; Büschges and Pearson, 1991). Stimulation of the hindwing tegula afferents is able to reset the flight rhythm at any phase of the wingbeat cycle by depolarizing the elevator motoneurons (i.e. finishing the downstroke; Wolf, 1993). Since tegula activity is correlated with the downstroke (see Fig. 2), this indicates that the tegula organs have an important role in determining the timing of activity in the elevator muscles within the wingbeat cycle.

The present study on freely flying animals not only corroborates previous observations concerning the effects of tegula deafferentation in fixed or restrained preparations (Büschges and Pearson, 1991; Büschges et al., 1992a,b) but also provides further evidence of how afferent input from the tegulae may be involved in the timing of elevator activity within the wingbeat cycle (Fig. 3). In individuals with ablated hindwing tegulae, phase values for elevator activity depended on the actual wingbeat frequency/cycle length. Under free-flight conditions, and in contrast to the apparent lack of functional significance of the forewing tegulae in previous studies of tethered flight (Büschges et al., 1992a,b), a significant correlation was also found after ablation of the forewing tegulae, although the effect was smaller than when the hindwing tegulae were removed.

Phase did not depend on wingbeat frequency/cycle length in sensory-intact animals or in animals in which all tegulae had been ablated. This indicates that the tegulae of both wings may synchronously participate in elevator timing, probably having an antagonistic influence since phase shifts in elevator activity are observed when only one tegula pair is lacking but not when both pairs are intact or have been removed. In addition, hindwing muscles can be phasically influenced by forewing

tegula nerve stimulation (Neumann et al., 1983). This supports our finding that, in the absence of the hindwing tegulae, the forewing tegulae can influence the motor patterns phasically.

No significant phase shift in elevator activity was observed after removal of all tegulae, as has been reported for tethered preparations (Büschges and Pearson, 1991). This may be due either to sensory open loops caused by tethering (e.g. Gewecke, 1975) or to the generally lower wingbeat frequencies that occur during tethered flight (Baker et al., 1981) allowing discrimination of such effects in more detail.

Role of the tegulae in the generation of aerodynamic output

A major result of the present investigation was the finding that, even after the removal of all tegulae, the locust is able to maintain free flight. Apparently, in spite of the absence of any afferent information from the tegulae, the flight system is able to generate aerodynamic output sufficient to support the body weight. This might be explained by the observation that the wingbeat frequencies of animals capable of free flight (irrespective of whether they are intact or have undergone tegula ablation) were not below the value determined as the probable 'minimum f_{WB} ' enabling level flight (i.e. sufficient aerodynamic output to support the body weight). However, other sense organs (e.g. frontal wind-sensitive hairs, stretch receptors) are still intact in animals with ablated tegulae, and it is probable that afferent information mediated by such organs is sufficient for the generation of an adequate output.

Only if all tegulae are removed is there a decrease in the maximum effective lifting power (Fig. 5). This is in agreement with a reduction in the range of observed ascent angle, α . Since P_{EL} is the aerodynamic output that has to be generated by all four wings when changing from level flight to climbing flight, we conclude from this observation that tegula ablation does not affect the basic aerodynamic output of the flight system that is required for free flight capability (even after removal of all tegulae, climbing flight was observed). The ablation of all tegula organs causes a reduction in the wingbeat frequency, and this may result in a decrease in the effective lifting power that can be additionally generated at maximum wingbeat frequencies. In tethered preparations, tegula removal seems to change the angular setting of the wing, and this is probably responsible for the decrease in the observed net lift generated (Wolf, 1993; but see Gee and Robertson, 1998).

Significance of the tegula for the regulation of flight speed

In the present study, the observed free flight speed of sensory-intact *S. gregaria* ranged from 3 to 6 m s^{-1} . This is in agreement with field studies reporting comparable flight speeds in *S. gregaria* (Waloff, 1972) and *Locusta migratoria* (Baker et al., 1981). The removal of the forewing tegulae had no significant effect on the mean flight speed. Ablation of the hindwing tegulae caused a shift in the flight speed towards significantly lower values, but this decrease was smaller than the reduction in speed following the deafferentation of all

tegula organs. The results obtained using the experimental approach described here, however, do not allow us to conclude that the tegula organs participate in the instantaneous regulation of the actual flight speed since, for the individuals investigated here, a wide range of flight speeds was still observed after ablation of all tegula organs. Therefore, the presence of tegula afferent information seems to be required to generate the motor output that enables maximum flying speeds, but it is apparently not obligatory for the thrust production that underlies flight capability (Zarnack, 1999).

Recovery of the motor pattern and behavioural parameters after tegula ablation

During previous studies of tethered flight, the capacity of the locust flight system to recover from tegula deafferentation was investigated. Following hindwing tegula removal, the flight motor patterns returned progressively towards normal in both mature (Büschges and Pearson, 1991; Büschges et al., 1992a,b) and immature locusts (Gee and Robertson, 1996). This recovery of the motor patterns was also observed in the present study (Fig. 7). This reorganization of sensory regulation has to be considered as functional because it corresponds to a full recovery of the free flight parameters: following hindwing tegula ablation, free flight speed (Fig. 6) and aerodynamic output returned to normal values within 2 weeks of ablation. No recovery in flight speed (Fig. 6) and aerodynamic output was found following ablation of all the tegulae. This also reflects the inability of motor patterns to recover after the removal of all tegulae, as previously reported for tethered preparations (Büschges et al., 1992a,b).

Our results are inconsistent with some recent findings: in tethered flying locusts, it was observed that recovery was less extensive when monitored by stroboscopic measurements of wingbeat frequency than when monitored using electromyography (Gee and Robertson, 1996). Investigating the free-flight ability (but not simultaneously the motor pattern) of locusts recovering from partial deafferentation, Gee and Robertson (1998) observed an overall deficit, which was independent of recovery from forewing or hindwing tegula ablation. In our opinion, this inconsistency is mainly caused by the large difference in the environmental temperatures at which the two studies were performed ($\Delta T=10^\circ\text{C}$). Gee and Robertson (1998) state that their experiments were carried out on 'a warm day (approximately 25°C)' compared with our investigations at 34°C . They report that a high proportion of control locusts were nonflyers. As shown in the present study, flight activity is strongly affected by the environmental temperature (see also Weis-Fogh, 1956a; Waloff, 1972). For the majority of *S. gregaria* investigated here, free flight activity occurred at temperatures above 27°C . We do not believe that the inconsistent findings are due to the fact that Gee and Robertson (1998) investigated *L. migratoria* rather than *S. gregaria*. Comparing the free flight behaviour of both of these locust species provided no significant evidence for any functional discrimination between either the free flight motor

pattern or the free flight parameters in either species (for details, see Fischer, 1998, pp. 72–74). Furthermore, tegula removal affects the wingbeat frequency in a similar manner for tethered flying *L. migratoria* (e.g. Büschges and Pearson, 1991) and for freely flying *S. gregaria* (see Fig. 3A).

In conclusion, it is clear that phasic information mediated by tegula afferents modulates the motor pattern during free flight in a manner similar to that previously reported from fixed or restrained preparations (Büschges and Pearson, 1991; Büschges et al., 1992a,b). Furthermore, the present state of knowledge on pattern generation during free flight is insufficient to conclude that there is an altered ‘basic’ mechanism of motor pattern generation during tethered flight. In contrast to previous assumptions and observations, the capability for free flight is maintained following tegula removal. This indicates that, even in the absence of tegula-specific information, motor patterns are generated that must be considered as ‘functional’ in the sense of being sufficient to maintain the capability for free flight. During free flight, the ultimate role of the tegula organs is apparently less important than predicted from tethered studies (Büschges and Pearson, 1991; Wolf, 1993). However, phasic information from a complete set of tegula organs is required for the motor output that enables ‘peak values’ of speed and effective lifting power to be achieved: when one set of tegula organs is ablated, the locust flight system is able to restore the pattern, including those free flight parameters that are for a time influenced by ablation.

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