

WING-BEAT COUPLING BETWEEN FLYING LOCUST PAIRS: PREFERRED PHASE AND LIFT ENHANCEMENT

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Summary

Pairs of locusts flying in tandem in a wind tunnel are known to couple their wing-beats intermittently. The rhythmically oscillating air flow from the front locust's wing-beat, detected by the rear individual, appears to convey the timing information for coupling. Three predictions of this arrangement were tested quantitatively in this study. (1) Given that the oscillating air flow has a wavelength of 7.5 cm, placing the rear locust 7.5 or 15 cm behind the front one should produce the same phase of coupling, whereas placing it at an intermediate distance of 11 cm should produce an opposite phase. (2) At any distance, the preferred phase at which wing-beat coupling occurs should depend, in part, on the difference in the wing-beat frequencies of the two locusts just before the coupling began. (3) At the moment that the wing-beats of the two locusts become coupled, a change should be observed consistently in the wing-beat frequency of the

rear individual only. Each of these three predictions was fulfilled.

We also recorded the instantaneous lift of the rear locust by tethering it to a laser torque meter. Lift varied with the phase of the wing-beats between the two locusts. For a given distance between the two locusts, lift was greater by a mean of 16% of the locust's body mass at those phases where coupling most commonly occurred than at opposite phases. This lift effect was seen even if the wing-beats of the two locusts drifted through these preferred phases without actually coupling. These results are discussed in terms of a possible energetic advantage conferred to the rear locust by flying in tandem and by coupling its flight rhythm to the leader's wing-beat.

Key words: locust, insect flight, phase, wing-beat, lift, *Locusta migratoria*.

Introduction

One of the most extreme examples of animal aggregation, in terms of numbers of individuals, is the locust swarm, which can include up to 10^{10} individuals and can extend for 10^3 km² (Cooter, 1989; Rainey, 1989). Within a given region of the swarm, locusts generally fly with their bodies parallel, and they can be separated by as little as 10 cm (Gunn *et al.* 1948; Rainey, 1976, 1989; Baker *et al.* 1984). Each locust's wing-beat produces a rhythmic flow field that extends behind its body for a distance of more than 30 cm (Kutsch *et al.* 1994), raising the possibility that others flying within this flow field will encounter turbulence inimical to stable flight.

In an earlier study, pairs of locusts flying in tandem in a wind tunnel were shown to couple their wing-beats intermittently, sometimes for periods of up to 1 min. The major sensory cue responsible for this wing-beat coupling appears to be the rhythmic flow field produced by the front locust's wing-beat and detected by the rear locust's cephalic wind-receptive hairs (Kutsch *et al.* 1994). Three procedures, deafening both locusts, blinding the rear locust combined with fixing its antennae in place and darkening the room, failed to prevent wing-beat

coupling; however, covering only the cephalic hairs did block coupling.

Further evidence that the rhythmic flow field was the cue responsible for coupling arose from measurements of the specific phase values at which coupling took place at different distances (Kutsch *et al.* 1994). Flying in a wind tunnel at 2 m s^{-1} , a locust with a wing-beat frequency of 19–20 Hz leaves behind a rhythmic flow field with a wavelength of approximately 7.5 cm. A tethered locust beating its wings at 20 Hz (and thus producing 20 waves of its flow field over 2 m) should produce a wavelength of 10 cm. However, the forces produced by tethered locusts are often much lower than those produced during free flight (Krüppel and Gewecke, 1985; Zarnack and Wortmann, 1989; Robertson and Johnson, 1993). For instance, lift is typically only 50–60% of body mass. If thrust is also lower when the locust is tethered, its wing-beat would slow down the air flow of the wind tunnel and thus produce a wavelength shorter than the expected 10 cm, which could account for the measured wavelength of 7.5 cm (Kutsch *et al.* 1994).

By placing the rear locust at multiples of this wavelength (7.5 or 15 cm) behind the front locust, a similar mean phase was obtained, whereas by placing it at an intermediate distance of 11 cm a nearly opposite phase was obtained (Kutsch *et al.* 1994). We will refer to this as the distance effect.

There were several uncertainties concerning the distance effect in the original study. First, as we had not specifically intended to study this effect, we did not systematically observe the same pair of locusts at different distances. Moreover, in those cases where we did change the distance for a given locust pair, doing so required that we open the side door of the wind tunnel, stop the flight, reposition the animals, restart their flight, and close the door. Therefore, only a small number of observations was made at each distance ($N=8$ for 7.5 cm, $N=5$ for 15 cm and $N=28$ for 11 cm). We regard the distance effect as significant, because it concerns the degree of adaptive integration that has evolved to permit coordinated group flight. Therefore, the present study includes a much more detailed investigation of the distance effect, under experimental conditions where the two animals could be moved relative to one another without opening the wind tunnel or stopping their flight. Our observations confirm our previous findings and describe the distance effect much more fully.

This more detailed examination has also permitted us to address an enigma concerning the range of phases of wing-beat coupling obtained for any given distance. The two locusts can be regarded as a pair of oscillators with unidirectional coupling, from front to rear. (In reality, the rhythmic flow field is not a simple oscillator, but has a complex, three-dimensional structure; Brodsky, 1991.) Physically, this simplified situation resembles the classic case of a *Zeitgeber* (an oscillator providing a rhythmic timing cue) and a responding oscillator whose timing is influenced by the *Zeitgeber* (Aschoff, 1963). The theory of coupled oscillators (von Holst, 1939; Wendler, 1974, 1983; Wilson, 1967) indicates that if the frequency difference (Δf) is very small between the *Zeitgeber* and the responding oscillator at moments when the latter is free-running (i.e. when the *Zeitgeber* is prevented from influencing it), the responding oscillator will become permanently coupled to the *Zeitgeber*. However, if Δf is somewhat larger, the coupling will be intermittent. Moreover, and of relevance here, the particular phase value at which the two oscillators will become coupled will depend upon the sign and size of Δf .

For a pair of flying locusts, the following complex situation can thus be predicted (Fig. 1). If, at any given moment just before the wing-beats of the two locusts become coupled, the front locust is flying with the higher wing-beat frequency (we will call this $+\Delta f$), when the wing-beats then become coupled, the rear locust's wing-beat will occur late relative to that of its front partner (Fig. 1A). If the front locust is initially flying with the lower wing-beat frequency ($-\Delta f$), then the rear locust's wing-beat will occur early relative to that of its front partner (Fig. 1B). If Δf is larger, then the deviation of the rear locust's wing-beat from simultaneity with that of the front locust will be greater (Fig. 1C). This latter effect will also occur if the strength of coupling is reduced.

Using rhythmical pulses of wind presented to the head of a single locust flying in a wind tunnel as a *Zeitgeber*, it has been shown that, by varying the *Zeitgeber* frequency relative to the locust's wing-beat frequency (i.e. varying Δf) by only ± 2 Hz, nearly a full range of coupling phases from 0 to 1 could be obtained (Horsmann *et al.* 1983; Wendler, 1983). A locust flying in a wind tunnel, and probably also in a swarm, alters its wing-beat frequency erratically within a narrow range of a few hertz. Thus, the Δf of a locust pair also varies erratically within this range; sometimes the front individual has the higher wing-beat frequency ($+\Delta f$) and sometimes the reverse ($-\Delta f$) occurs. It could, therefore, be expected that a pair of locusts, at any separation distance, would adopt widely varying phases of wing-beat coupling.

The enigma mentioned above is that, in spite of this prediction of widely varying coupling phases, coupling appears to occur over a restricted range of phases for any given locust separation (Kutsch *et al.* 1994). In the present study, we investigate this enigma. We show that, under natural conditions, although Δf affects phase in the predicted direction, these effects are sufficiently small that phase values at a given locust separation remain quite consistent. This results, in part, from the fact that coupling only occurred when $+\Delta f$ or $-\Delta f$ was small.

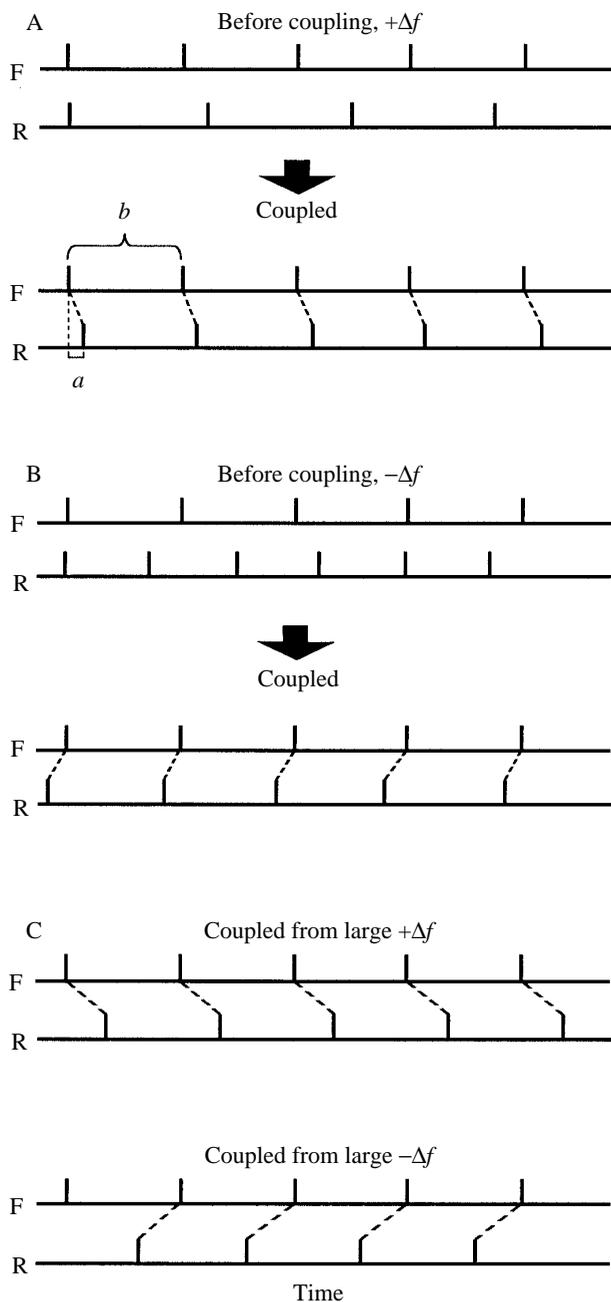
We also investigated the adaptive advantage of phase coupling. Given that locusts are very strong fliers, with swarms travelling without rest for up to 60 h (Johnson, 1969), and that they have several specializations that are known to improve flight efficiency (Nachtigall, 1989; Möhl, 1989), we considered the possible energetic advantage produced by wing-beat coupling. Specifically, as lift accounts for approximately 95% of the force produced by a flying locust (Jensen, 1956), we investigated whether coupling positively affects the lift production by the rear locust. We report a consistent lift boost to the rear locust during periods of wing-beat coupling.

Materials and methods

For all experiments we used *Locusta migratoria, phasis gregaria*, at least 2 weeks after their final moult. The experiments were carried out at two different locations and thus on slightly different systems. Experiments on the distance effect and on the influence of Δf on phase were carried out in Jerusalem, and those involving lift measurements were carried out in Köln.

The distance effect and the influence of Δf

In these studies, the front locust was tethered from above at the pronotum and the rear locust from below at the meso-metathoracic sternum, so that there was no obstruction to the flow field arriving at the rear locust's head hairs and wings. The tarsi of the front locust's legs were removed, as were the entire front and middle legs plus the rear tarsi of the rear locust, to prevent the tarsal reflex from stopping flight. The animals were placed inside a large laminar-flow wind tunnel described previously (Kutsch *et al.* 1994). The air speed of 2 ms^{-1} ,



measured with a hot-wire anemometer (800 VTP, Datametrics, Watertown, MA), is within the range of relative wind speeds that the animals would experience in free flight.

For this study, we modified the wind tunnel in the following ways (Fig. 2). The rod to which each locust was tethered was connected to a separate horizontal arm. Each arm passed through a slit in the side wall of the tunnel, which permitted the locust to be moved independently from outside. The rear locust could be moved horizontally and the front locust vertically. Each could be moved closer to or further from the centre of the tunnel and could be rotated in the pitch plane. In these experiments, the long axes of the bodies of both locusts were always in the same vertical plane, although the rear locust

was always below the front individual, at an angle of 25° , roughly the angle of maximal flow-field air displacement (Kutsch *et al.* 1994). The pitch of each locust's body was kept at roughly its natural angle during flight (7° , head upwards).
 During an experiment, we first positioned the two locusts 7.5 cm apart and allowed them to fly for 15 min without disturbance, to permit wing-beat frequencies to stabilize and the normal flight posture of the legs to be assumed by both locusts. Then, for a period of up to 30 min we recorded electromyograms (EMGs) (see below). The distance between the locusts was then altered and recording was continued for a similar period, until each distance had been investigated. The distances used were 7.5, 11 and 15 cm (head-to-head); the sequences were varied and the same distances were sometimes repeated. Body length is typically 5 cm. Given that the wavelength of the rhythmic flow field is roughly 7.5 cm (Kutsch *et al.* 1994), the flow events at 7.5 and 15 cm are in phase with one another, and those at 11 cm are nearly fully out of phase with events at the other two locations. The actual flow field, however, is much more complex than a single sine wave (Brodsky, 1991).

Electromyograms (EMGs)

The same recording methods were used at both research sites. To determine the instantaneous phase between the two locusts, we recorded electromyograms (EMGs) from a hind-

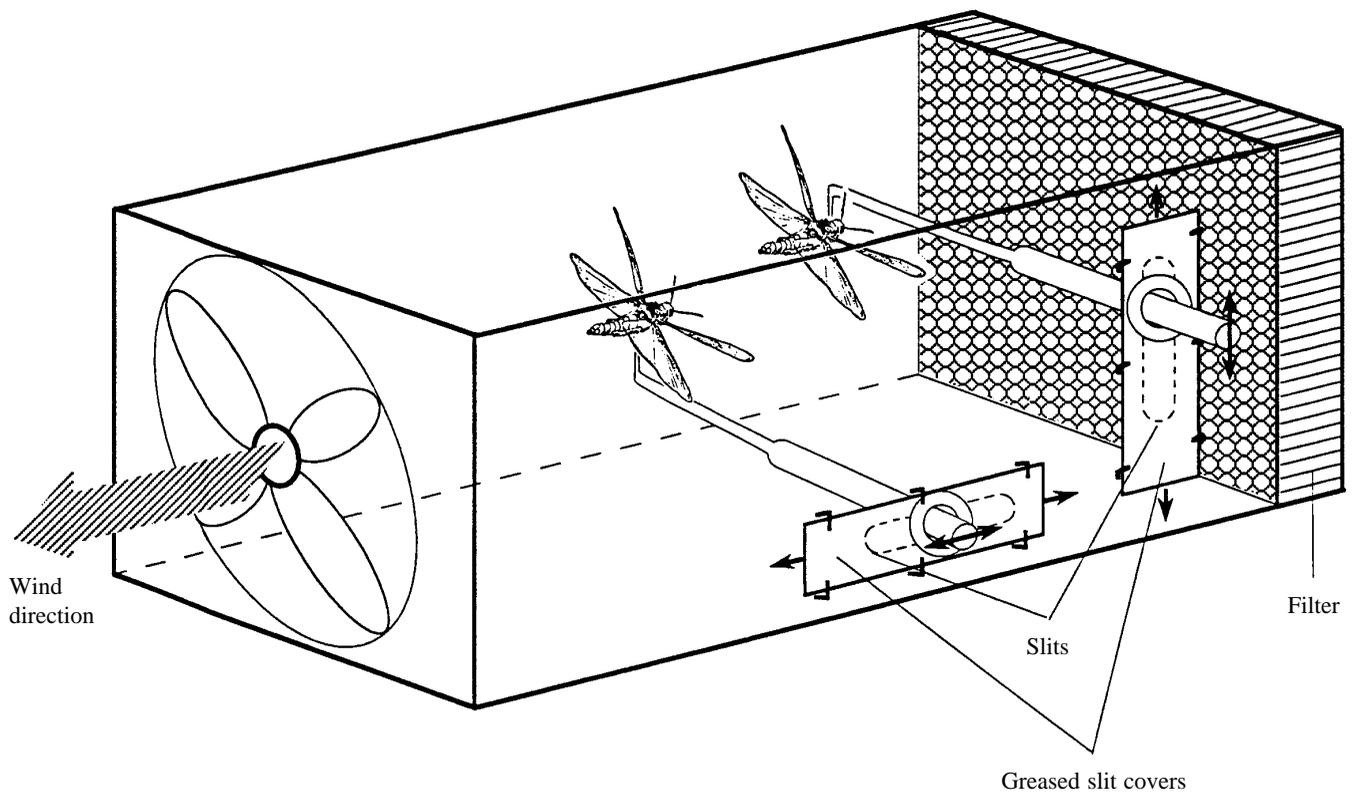


Fig. 2. The wind tunnel showing two locusts flying in tandem. Each locust can be moved independently from outside the tunnel during flight. The arm to which each locust is attached passes through a slit permitting movement in one direction (vertical for the front locust and horizontal for the rear one). Air flow through the slit is prevented by a greased plate that slides together with the arm. Not drawn to scale (actual tunnel dimensions, 60 cm high \times 60 cm wide \times 100 cm long). See text for further details.

wing subalar muscle of each animal. This is a wing depressor muscle, M129 (Snodgrass, 1929). We implanted, in each muscle, a pair of silver wires insulated to their tips, 70 μm in diameter, and secured the wires to the cuticle with wax. The signals were a.c.-amplified (Grass P15 amplifier) and stored on tape (Hewlett-Packard 3960 instrumentation tape recorder) for further processing off-line, in Jerusalem by the program Computerscope (RC Electronics, Goleta, CA) plus locally produced programs, or on locally produced programs in Köln.

Following an experiment, we identified periods of clear phase coupling by triggering the oscilloscope sweep with the EMG signals from one locust and recording the signal from the other locust on this trace, at a sweep rate that was sufficiently low to include at least one entire wing-beat cycle. Sections in which the EMG record remained in a stable position at any location on the screen were then selected for analysis. In practice, if the EMG did not stabilize on the screen, the value of Δf was usually rather high, apparently making it impossible for coupling to occur (Horsmann *et al.* 1983; Kutsch *et al.* 1994).

EMG data were processed by plotting, as functions of time, the phase of the EMG of the rear locust in the cycle of the front locust, the period duration ($1/\text{wing-beat frequency}$) for each animal, the number of EMG spikes per cycle for each animal and the lift of the rear locust (see below).

Lift measurements

In these studies, we used only male locusts, both placed in front of a laminar-flow wind tunnel operating at 1.8 m s^{-1} (measured with a Heinecke anemometer). Head-to-head distance was 8.5 cm, with the rear locust again positioned below the leader, at an angle of 25° . Both locusts were tethered from above at the pronotum. We removed the metathoracic legs from both locusts, as otherwise they tended to grasp the tethers, inhibiting flight, or to interrupt the laser beam from the torque meter.

The rear locust was tethered to a laser torque meter oriented so as to register the relative lift of the rear individual. This device has been described previously (Dombrowski, 1991; U. Dombrowski and G. Wendler, in preparation). Briefly, a laser beam was projected onto a small mirror attached to the tether such that upward movements of the locust (in the range of micrometres) produced a greatly magnified deflection of the reflected beam. The reflected beam was projected onto a position-sensitive diode located 1.5 m from the locust. The device could detect changes in lift produced by a single wing-beat; however, we typically used a 5 Hz low-pass filter, which largely eliminated the effects of single wing-beats. The mechanical resonance frequency of the system, with a locust in place, was roughly 300 Hz, sufficiently high not to affect our lift measurements. Lift output was linear, at least within the

range 0–2 g, which is about 25 % more than the mean mass of the locusts used here. Changes in thrust also produced an output, but the device was 10–20 times more sensitive to lift forces.

Statistical analyses

Data concerning the distance effect and the effect of Δf on phase are presented as phase histograms, with mean values computed using circular statistics (Zar, 1974). It is not correct to cross-compare different phase histograms, since the phase value sampled with each wing-beat is not independent of the preceding or later values. Thus, for most of the data presented, we selected those instances of wing-beat coupling that were separated by at least one full continuous sweep of phase which drifted through all phase angles. We regarded any two such coupling intervals as independent samples, since the system had the possibility of coupling at any of the intervening phases. Our criterion that coupling had occurred was at least 0.75 s (which equals approximately 13 wing-beats) with less than ± 0.05 fluctuation of phase. We judged the conclusion of coupling as the moment at which there was a clear onset of phase shifting. If this terminal phase shifting was in the direction opposite from that prior to the coupling (i.e. the sign of Δf was reversed), then, although we included this as a *bona fide* coupling event, we did not use the data in comparisons between phases of couplings with positive Δf versus those with negative Δf .

In total, 239 episodes of phase coupling fulfilled these criteria and were analyzed. For these data, we constructed phase histograms for each distance setting of each locust pair and computed their mean values (and dispersion, using circular standard deviation; Zar, 1974), expressed in phase units from 0 to 1. Phase is a circular measure, with 1 equal to 0. However, because phase was plotted as a function of time, one can consider all possible phase values within the first wing-beat cycle sampled to be from 0 to 1, those in the second cycle to be from 1 to 2, etc. In particular, an event occurring after the end of one cycle can be thought of as having a phase greater than 1. In some cases, we found it useful to consider phase values higher than 1. Statistical comparisons were carried out among the different data sets, using both the nonparametric circular statistics test Watson U^2 (Zar, 1974) and a Mann–Whitney U -test.

Results

The distance effect

We analyzed over 32 000 wing-beat cycles in four pairs of locusts. For two pairs, results were obtained for only two distances, repeated in the sequence 7.5 cm, 11 cm, 7.5 cm, 11 cm, for a third pair in the sequence 7.5 cm, 11 cm, 15 cm, 7.5 cm, 11 cm, 15 cm, and for the fourth in the sequence 7.5 cm, 11 cm, 15 cm. A previous study (Kutsch *et al.* 1994) found relatively high and similar phase values for separation distances of 7.5 and 15 cm (0.61 and 0.63 respectively) and a much lower value for 11 cm (0.35). However, these three phase

values were based on a small sample size, and did not include systematic tests of the same locust pair at different distances.

Fig. 3 shows phase histograms for two pairs of locusts at different separation distances. In Fig. 3A, the top panel represents all the data, including values that did not meet our coupling criteria, for a 4.5 min period of flight. This flight contained 12 couplings that met our criteria, and showed phase drifting between them. The second panel shows only the phases during the 12 criterion couplings for the same data. The peaks are similar in the two histograms; however, owing to the background phase drifting present in the top panel, its mean phase (arrow) is shifted relative to that of the second histogram (a shift of 0.22 phase units to the right, from 0.89, through 1.0, to 0.11). As discussed previously, phase is a circular measure, and the shift here could therefore also be 0.70 to the left; however, as the shapes of the curves are similar (Fig. 3A), it is clear that the smaller shift is more appropriate.

A similar reduction in background phase drifting (not shown) with minimal change in the shape of the main peak was found for all separation distance and all locust pairs. For the four locust pairs, the criterion couplings accounted for 9 %, 12 %, 19 % and 26 % of the flight durations, respectively (overall mean 17 %). These values are likely to under-represent the true proportion of coupling, since the overall sample time usually included several periods in which one locust stopped flying, followed by flight initiation. During these periods and for a while thereafter, the difference in wing-beat frequencies was usually sufficiently great to preclude coupling.

Considering only the data from criterion couplings, Fig. 3A shows that the mean phase value at 11 cm is drastically shifted, to 0.40. (A second peak, near a phase value of 0.9, was found here and on one other occasion for the 11 cm distance, out of a total of seven data sets.) At a distance of 15 cm, the mean phase was 0.91, very similar to that at 7.5 cm.

Fig. 3B shows the phase histograms for a second locust pair. Similar mean phases were obtained at 7.5 cm (0.90 and 0.87) and at 11 cm (0.31 and 0.41).

Fig. 4A summarizes, for each distance setting and each locust pair, the overall mean phase and circular standard deviation for all 239 occasions of wing-beat coupling, irrespective of the durations of the couplings. Both within and among locust pairs, the mean phase values fell into two roughly opposite ranges: i.e. 0.28–0.48 for a separation of 11 cm, and 0.78, through 1.0, to 1.08 for both 7.5 and 15 cm separations. As mentioned above, an event occurring after the end of one cycle can be thought of as having a phase greater than 1. In this case, a phase of 1.08 is logically equivalent to 0.08. It is clear, however, that the data in Fig. 4A for 7.5 and 15 cm separations form a cluster that is far better represented by the range of phases from 0.78, through 1.0, to 1.08 than from 0.78 down to 0.08.

The scatter was generally smallest for 7.5 cm separations, although there were exceptions. Neither the pattern of phase values nor that of the scatter is likely to be due to fatigue during the experiment or to other time-dependent factors, as shown by the similar results obtained for repeats of each separation; for

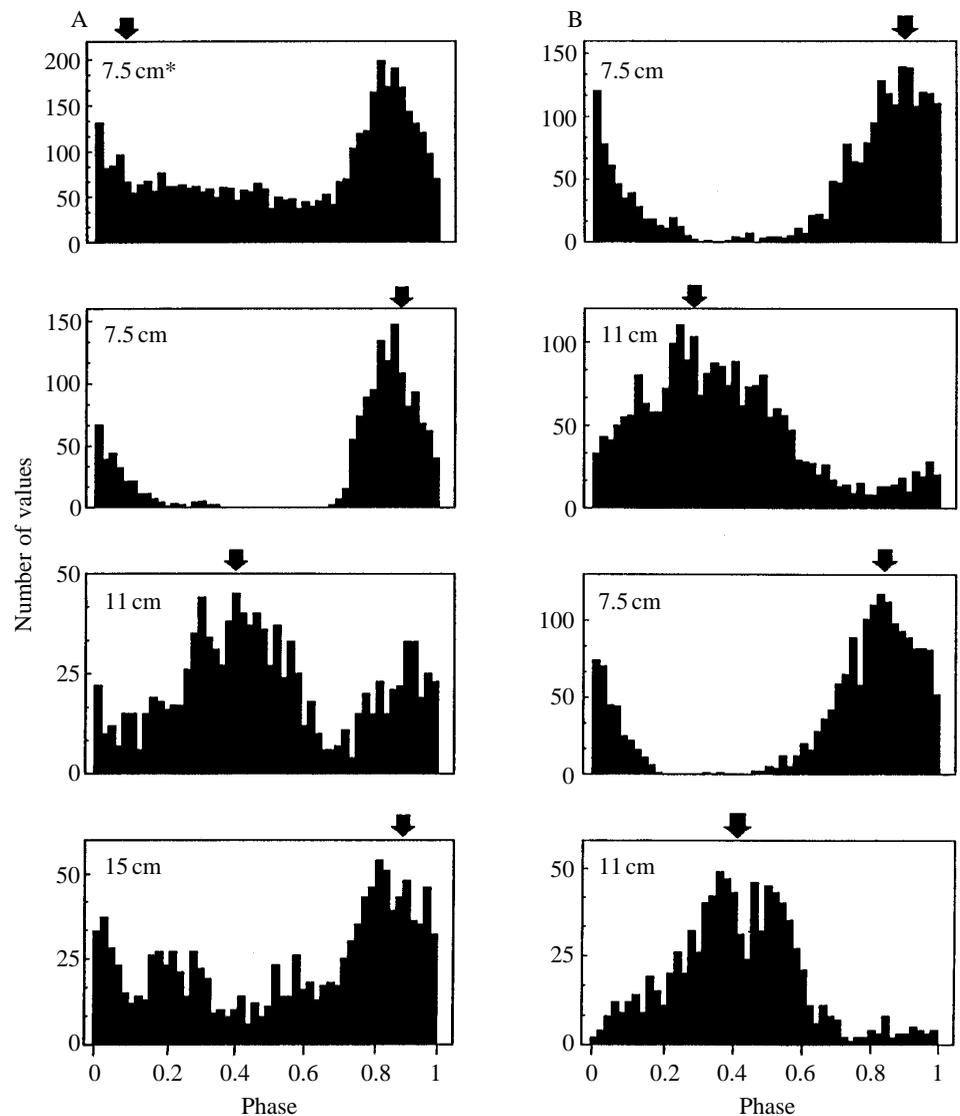


Fig. 3. Phase histograms for two pairs of locusts at different separations. Arrows indicate mean phase values. (A) One locust pair at 7.5, 11 and 15 cm separation distance. The top panel (7.5 cm*) shows data from all wing-beats in a 4.5 min flight sequence. All other panels of this figure give data only from wing-beat couplings meeting our criteria. The major peaks are similar for 7.5 and 15 cm, but nearly opposite for 11 cm. Mean phases (\pm circular standard deviations), from top to bottom, are 0.11 ± 0.24 , 0.89 ± 0.10 , 0.40 ± 0.29 and 0.91 ± 0.25 . (B) A second locust pair placed at each of two separation distances, in the sequence shown. Mean phases, from top to bottom, are 0.90 ± 0.14 , 0.31 ± 0.21 , 0.87 ± 0.13 and 0.41 ± 0.18 .

example, in locust pairs b, c and d. Possible sources of scatter are considered later.

The statistical analysis presented in Fig. 4B (top panel) shows that the phases at 11 cm distance were highly significantly different from those at either 7.5 or 15 cm in the same pair of locusts (in all cases using the Mann–Whitney U -test and in all but two cases using the weaker circular statistics test). Using circular statistics, there was no significant difference, within any locust pair, between 7.5 and 15 cm, or between successive returns to the same distance (Fig. 4B). Using the Mann–Whitney U -test, just one of these comparisons showed a significant difference.

As the flow field from the front locust arrives at the sensory hairs on the head of the rear locust only after a certain period, it may be more meaningful to determine the phase of the rear locust relative to a previous wing-beat cycle of the front locust, rather than the present one. For a head-to-head distance of 11 cm, assuming that the flow field travels at 2 m s^{-1} , the flow field arrives approximately one wing-beat cycle later at the head of the rear locust. We therefore recomputed the phase, for

a single data set at 11 cm, using a single wing-beat cycle earlier for the front locust. We assumed that the front locust's present cycle had the same duration as the prior one. We then obtained phase values in the range 1–2 rather than 0–1. Subtracting 1 from each value, we obtained phase values that could be compared with our normal data. There was no noticeable difference in the data obtained using this method. We also calculated phase relative to the fifth and the tenth earlier wing-beats of the front locust, and again found no obvious differences.

In summary, the phase of wing-beat coupling depends consistently on the distance between the locusts and repeats with multiples of the wavelength of the front locust's flow field. Scatter was generally least for 7.5 cm separation.

Effect of Δf on phase

As discussed earlier, the phase at which coupling occurs between the wing-beats of a pair of locusts should depend on Δf . If, prior to coupling, the rear animal has the lower frequency (i.e. for $+\Delta f$), it should couple later, relative to the

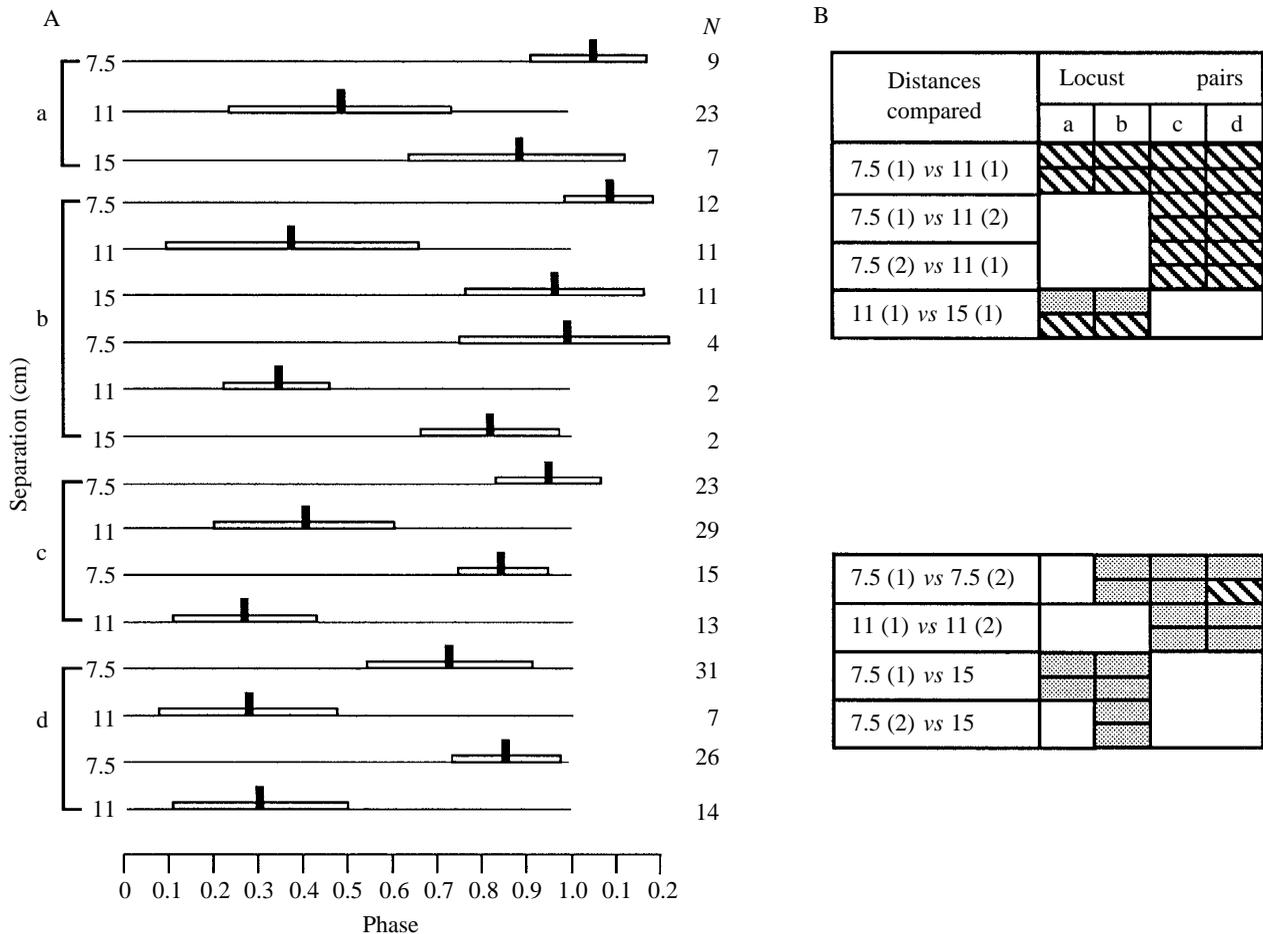


Fig. 4. Summary of data on the distance effect. (A) Means plus circular standard deviations of all data sets for all four locust pairs, a–d. Head-to-head separation of the locust pairs given in cm. N is number of criterion couplings. The data shown in Fig. 3 are the first three distances from b, and four data sets from c, but the mean values here are slightly different as they represent the overall means, i.e. for each coupling event, we determined the mean phase and finally calculated the overall mean. Fig. 3, by contrast, represented the running mean of all wing-beats of all criterion couplings and, hence, the durations of the different couplings influenced the means calculated. (B) Summary of statistical comparisons. For each separation distance and each locust pair, the results of two statistical tests (circular statistics and Mann–Whitney U -test) are shown in the upper and lower rectangle, respectively. Hatching indicates $P \leq 0.01$, shading indicates $P > 0.05$. White regions indicate no data available. Upper panel, comparisons between 11 cm and either of the other two separation distances. The numbers 1 or 2 in parentheses refer to the first or second data set obtained from a locust pair at the separation distance indicated. Bottom panel, comparisons between the first versus second data sets for a given locust pair at a given distance, e.g. 7.5 (1) versus 7.5 (2), or at multiples of the flow field wavelength, i.e. 7.5 versus 15 cm. (Data from the last two lines of locust pair b in Fig. 4A, each of which is represented by only two data points, were not included in the statistical tests.)

front animal, than if it has the higher frequency ($-\Delta f$) (see Fig. 1). Note that, although the rear animal in Fig. 1A clearly coupled later than that in Fig. 1B, on a phase histogram, the phase in Fig. 1A would be low (approximately 0.12) and that in Fig. 1B it would be high (approximately 0.88). However, $+\Delta f$ will result in the coupling moving to the right and $-\Delta f$ will result in it moving to the left, relative to the synchronizing event from the *Zeitgeber*.

We studied the effect of Δf on phase by separating the phase data, for a given pair of animals at a given distance, into episodes in which, prior to the coupling, there was $+\Delta f$ and those where there was $-\Delta f$. Fig. 5 shows a histogram for one locust pair at a distance of 11 cm. All $+\Delta f$ trials together gave a mean phase of 0.48, a shift to the right of the mean

for all $-\Delta f$ trials (0.31). In each of six different sets of data (at 7.5 or 11 cm, from all four pairs of locusts) where there were sufficient values to make a comparison, the mean phase was higher for $+\Delta f$ than for $-\Delta f$. The mean phase difference between $+\Delta f$ and $-\Delta f$ for all six data sets was 0.33, and was significant ($P = 0.015$, Wilcoxon rank test). Although there was a shift to the right for $+\Delta f$ versus $-\Delta f$ couplings, this shift was sufficiently small that most of the coupling phases were restricted to approximately half the range from 0 to 1.

Can this relatively small change in phase be explained on the basis of a relatively small range of Δf values just prior to the start of coupling? To test this, we measured the absolute value of Δf just prior to coupling, at distances of 7.5 and 11 cm

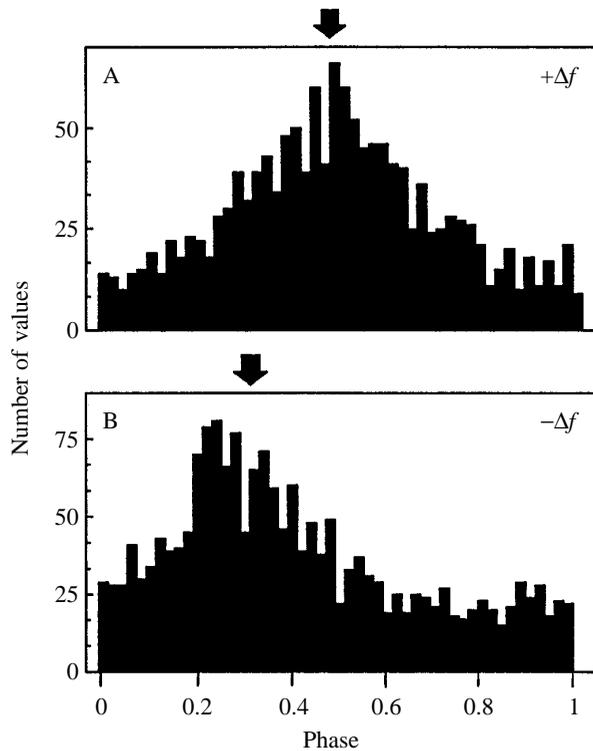
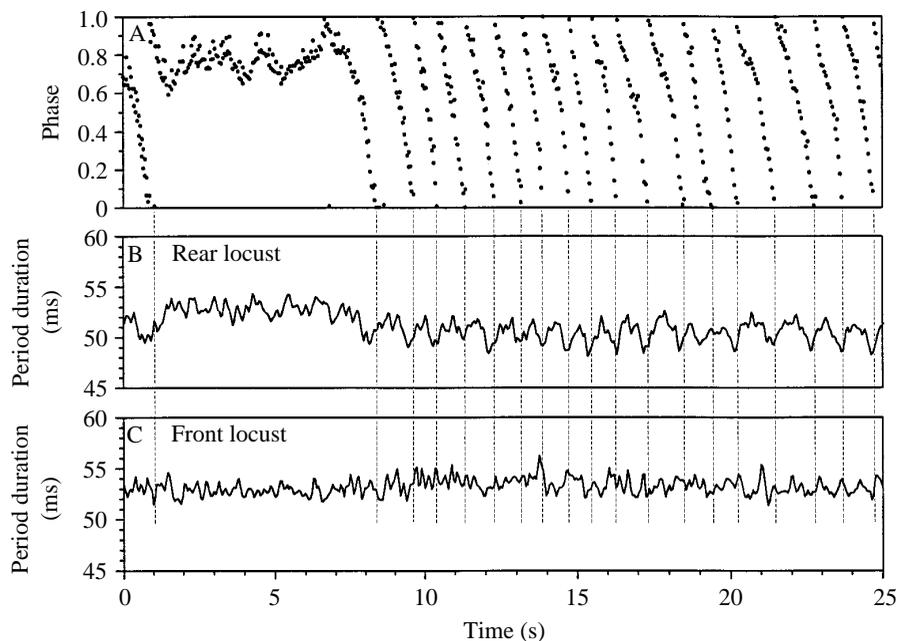


Fig. 5. Phase histograms from one locust pair separated by 11 cm. (A) $+\Delta f$. Mean phase is to the right of the mean phase for the histogram in B, which is for $-\Delta f$. Data are from the entire flight sequence, not just the criterion wing-beat couplings, and are from Fig. 4A, coupling b. Arrows indicate mean phase values, 0.48 in A and 0.31 in B.

in two locust pairs, and obtained the low value of 0.9 ± 0.32 (mean \pm S.D., $N=59$).

In an earlier study, in which a single locust, flying in a wind tunnel, coupled its wing-beat to the rhythm of repeated, controlled wind puffs injected into the wind stream (Horsmann

Fig. 6. Phase and period duration of the wing-beats of each locust as a function of time. (A) Each point represents the phase, for a single wing-beat cycle, of the wing-beat of the rear animal in that of the front animal. Initially, there is a long period of wing-beat coupling at a phase of approximately 0.8, followed by phase drifting with a slight tendency to couple between phase values of 0.5 and 1. (B) The period duration of the wing-beat of the rear locust varies systematically with phase, with higher values corresponding with higher phase values. (C) The results for the front locust show no such variation with phase. Since the rear locust's phase progressively decreased during phase drifting (that is, its wing-beat comes progressively earlier relative to the front locust's wing-beat, a case of $-\Delta f$), an increase of the rear locust's period duration is in the correct direction to cause partial or full wing-beat coupling, as is seen here.



et al. 1983), the peak puff speed used was 0.7 m s^{-1} peak-to-peak. This is close to the fluctuations in air speed produced by the front locust in our study (approximately 0.9 m s^{-1} peak-to-peak, at the position of the head of the rear locust at a separation of 11 cm; Kutsch *et al.* 1994). Given this similarity, we were able to compare our data with those of the earlier study, although the specific parameters for the wing-beat coupling have not been identified. To do this, we multiplied our mean Δf (0.9) by the absolute value of $\Delta \text{phase}/\Delta f$, obtained from the prior study (Kutsch *et al.* 1994), to obtain the mean phase difference that should be produced by the Δf values in our experiment, assuming that the two experimental situations were similar. This calculation gave a value of 0.095 phase units per 1 Hz change in Δf . Thus, the mean range of Δf in our experiments, ± 0.9 Hz, should produce a mean phase difference of ± 0.086 , or twice this amount, namely 0.17 for $+\Delta f$ versus $-\Delta f$. The mean phase difference we actually obtained for $+\Delta f$ versus $-\Delta f$ was 0.33, larger than the predicted value. Thus, although the low mean Δf prior to wing-beat coupling can contribute to the small variation in phase that we recorded, it is presumably not the only determining factor.

Which locust is the Zeitgeber and which the responder?

In the experiments carried out in Köln, we used a different wind tunnel, operated at a slightly lower wind speed (1.8 m s^{-1}), and used male locusts instead of females. Nevertheless, we obtained wing-beat coupling. With the locusts separated by 8.5 cm, the mean phases were similar to those in the Jerusalem studies with a 7.5 cm separation (Figs 6–9). Each of three pairs of locusts that flew for long periods showed wing-beat coupling in a consistent range of phases, reconfirming the existence of this phenomenon.

Using these data, we tested the prediction that it is the rear locust that alters its frequency to achieve wing-beat coupling. If so, the period duration of the wing-beat of the rear locust

should vary with phase. Such a result would be consistent with, and thus would help to confirm, the observation that the rear animal, sensing the front locust's rhythmic flow field, adjusts its own wing-beat frequency to couple to the front animal's wing-beat (Kutsch *et al.* 1994).

In Fig. 6, a single episode of wing-beat coupling is shown, followed by several downward sweeps. A downward sweep indicates a $-\Delta f$, since the phase of the rear locust's wing-beat relative to that of the front one becomes earlier with each successive wing-beat. On most of these sweeps, the concentration of points is greater for phases higher than 0.5, indicating a slight decrease in Δf but not complete coupling of the wing-beats. The corresponding period durations of the wing-beats of the locusts are also shown in Fig. 6. During the initial long coupling episode, the rear locust increased its period duration while that of the front animal remained constant. Moreover, throughout the record, the period duration of the rear locust varied consistently with phase, while no such pattern could be detected for the front locust. These results are presented graphically in Fig. 7, which shows that the period duration of the rear locust increased for those phases at which coupling occurred.

We further investigated, from the Jerusalem data, 61 instances of wing-beat coupling in two pairs of locusts at 7.5, 11 and 15 cm separations and determined whether the rear locust, the front one or both locusts had altered their period durations in establishing wing-beat coupling. In 41 instances, the rear locust altered its period duration in the appropriate direction to establish coupling; in 10 instances the front locust did so, and in 10 cases both locusts did so together. These data are significantly different from random ($P < 0.0001$, χ^2).

Lift measurements

Fig. 8 shows a segment of flight that included two periods of wing-beat coupling, each lasting for approximately 2 s, several phase sweeps with little or no coupling and the simultaneously measured relative lift of the rear locust. The upward deflections in lift correspond with phase values greater than 0.5, including intervals in which phase coupling did not occur. (Three such sequences are indicated by asterisks on Fig. 8.) As can be seen, each shaded interval shows an upward lift deflection. The lift fluctuations could follow phase values drifting at a rate of approximately 1 full phase cycle per second (and in other examples, at twice this rate). We also found that the increased lift could persist without decay during more prolonged wing-beat coupling (see far left shaded region on Fig. 8).

Fig. 9 summarizes the data obtained from a flight sequence from a different locust pair. The difference in the mean lift, from the low values at a phase of approximately 0.2 to the high values at a phase of approximately 0.7 was 1.8 mN (which is 0.18 g or 16% of the mass of a 1.15 g male locust). The difference between the lowest and the highest actual lift measurements was 4.1 mN (which is 36% of body mass). We obtained similar results for all three pairs of locusts that flew well for prolonged periods. As the output of the lift meter

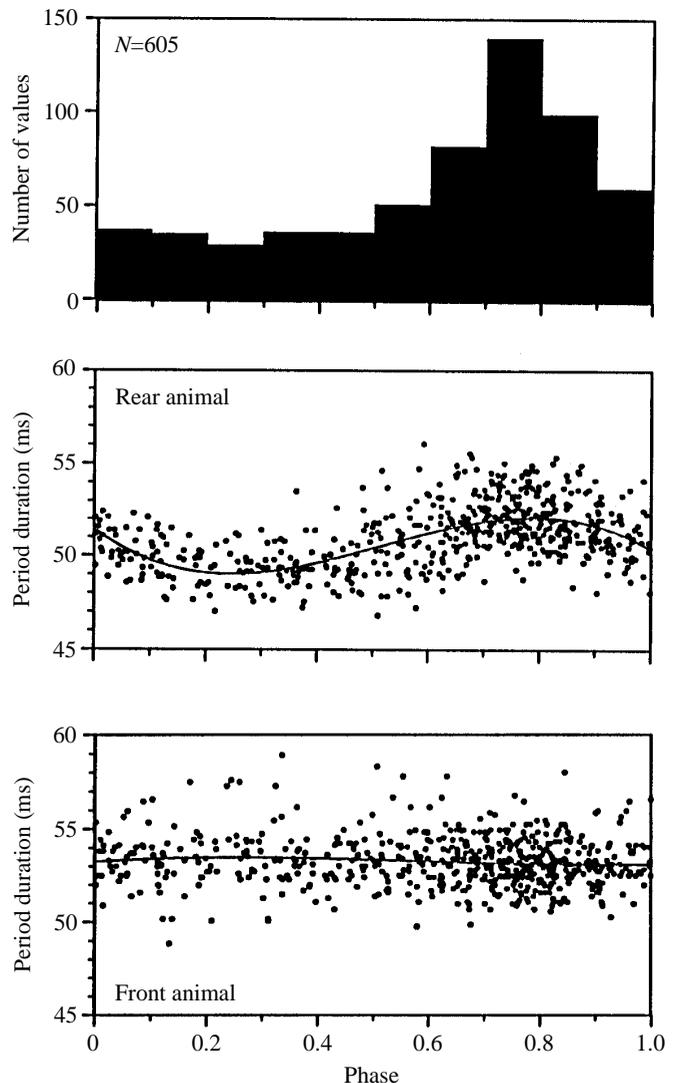


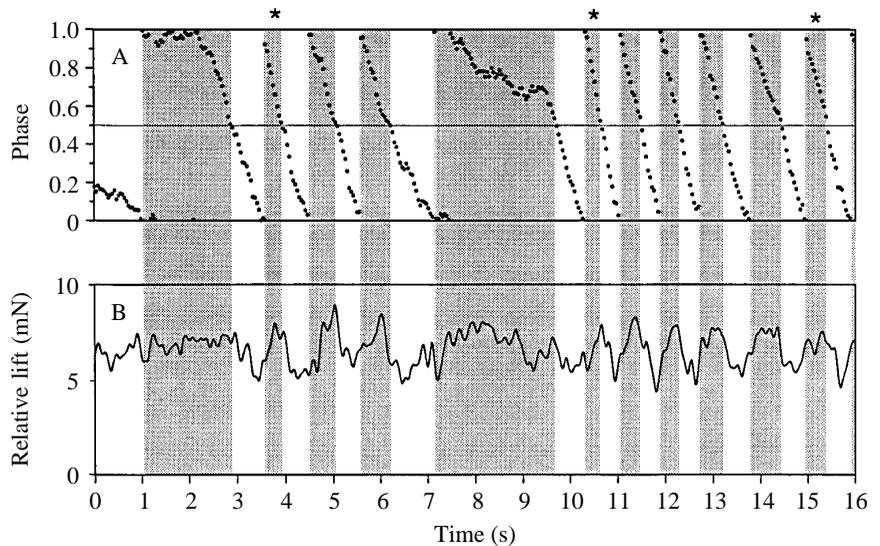
Fig. 7. Wing-beat period duration as a function of phase, based on the data shown in Fig. 6. (A) Phase histogram, showing a peak at a phase of approximately 0.8. (B) Period duration of the rear locust shows a peak at approximately the same phase, while that of the front animal (C) shows no such peak. Curve fitting was by a third-order polynomial regression.

drifted over the course of several minutes, it was not possible to compare systematically the lift production of an individual when flying alone or in tandem.

A further complication for a locust flying in the wake of another individual is that it will encounter the net downward air flow produced by the leading locust and will therefore receive net a net downward push. In order to determine whether this downward flow or the phase-dependent lift dominates in determining the overall lift experienced by the rear locust, we measured the effect of the downward flow on the lift of the rear locust. In four pairs of female locusts, at different separation distances, we compared the lift of the rear locust during the brief periods when the front locust flew and the intervening periods when it did not.

Fig. 10A shows an example with the rear locust 10 cm

Fig. 8. Phase and relative lift as a function of time. (A) Phase of the rear locust during 16 s of flight. Each point represents the phase for a single wing-beat cycle. In this sequence, two periods of wing-beat coupling, each lasting a few seconds, and several episodes of phase drifting are shown. Phase values between 0.5 and 1 are shaded. (B) Relative lift of the rear animal. The lift was higher for phase values between 0.5 and 1 than between 0 and 0.5. The asterisks indicate three instances of phase drifting that showed no sign of wing-beat coupling. See text for further explanation.



behind and 5 cm below the front locust. At first, neither locust is flying. As the rear locust initiates flight its lift trace increases

by approximately 8 mN, equal to 62 % of its body mass. After approximately 2 s the front locust made two brief flights, as shown by its EMG record. Each of these resulted in a reduction in the rear animal's lift (the first was equal to 12 % of the body mass, the second slightly less, measured from the first downward peak), followed by a return to previous levels each time the front animal ceased to fly. In a total of nine such trials in two pairs of locusts with a 10 cm separation, the mean lift reduction at the onset of flight by the front locusts was 13 % of the rear individual's body mass for one locust pair and 8 % for the other pair. In a third locust pair, separated by 20 cm, a lift reduction of 11 % of body mass was measured. In a fourth locust pair, at 16 cm separation, there was a reduction in lift only when the rear locust was flying at the time that the front locust began a bout of flight (Fig. 10B). When the rear locust was replaced by a dead one, with its wings waxed into a mid-downstroke position (Fig. 10C), the mean lift reduction upon the start of flight by the front locust was similar to that for a flying rear locust. This suggests that at least part of the lift reduction caused by the front locust may be due to passive forces produced by the downward flow of air.

Discussion

In this paper, we have confirmed the effect of separation distance on the phase of wing-beat coupling in pairs of locusts. This finding thus strengthens the contention from a previous study (Kutsch *et al.* 1994) that wing-beat coupling is determined by the rear locust from sensory information about the flow field from the front locust, as similar phase values are found for coupling at multiples of the wavelength of the flow field (7.5 cm *versus* 15 cm) and intermediate values at an intermediate distance (11 cm).

There is considerable scatter in the phase measurements at each distance sampled, for each locust pair (Figs 3, 4). One possible source of this scatter is the complex, three-dimensional nature of the wake of a flying insect (Brodsky, 1991). Thus, the wind-receptive hairs of the rear locust receive

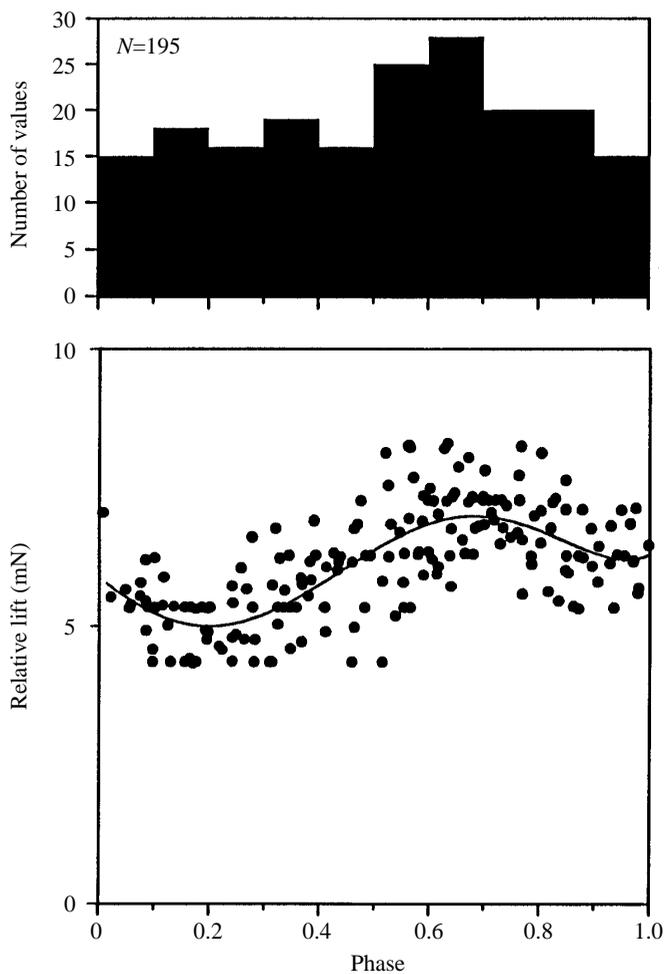


Fig. 9. Relative lift of the rear locust as a function of phase. The data are from a different locust pair from that of Fig. 8. (A) Phase histogram. (B) Relative lift as a function of phase. See text for further details.

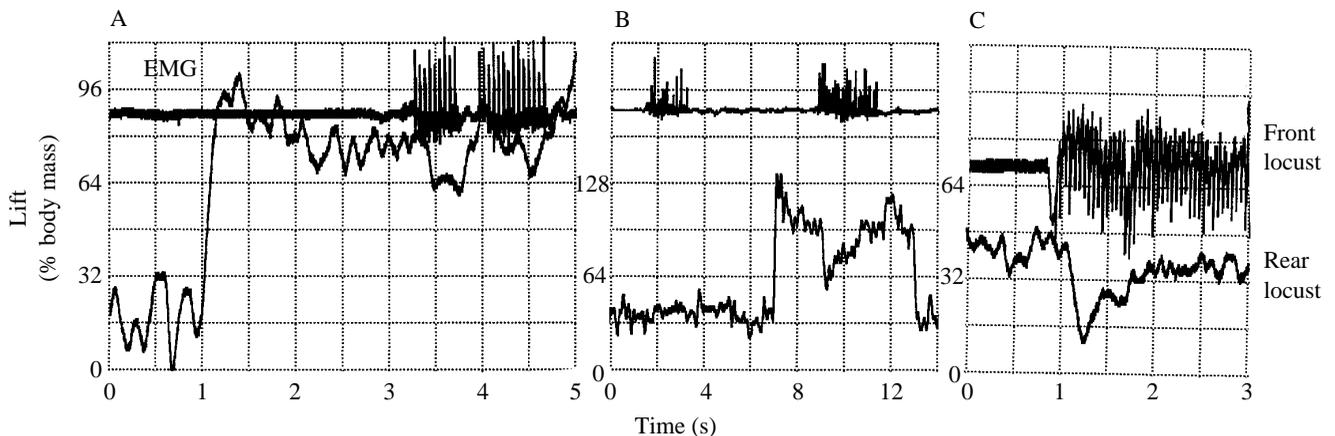


Fig. 10. The effect of the onset of flight in the front locust on the rear locust's lift. (A) The rear locust begins a bout of flight, shown by the upward deflection of its lift record. The EMG record for the front locust shows two flights, each of which reduces the lift of the rear locust. (B). The EMG record for the front locust shows two flights, the first while the rear locust was not flying and the second after the rear locust had begun to fly. (C) The onset of flight by the front locust affects the lift of a dead rear locust, with wings set with wax into the mid-stroke position. The distance of the rear locust behind/below the front locust is (in cm:) 10/5 for A and C and 16/6 for B. All locusts were females. The mass of rear locust was 1.3 g in A and 1.55 g in B.

a signal whose velocity and direction are complex functions of time, rather than a pure sine wave. A second source of scatter arises because even slight differences in the wing-beat frequency of the front locust, fluctuations in its forewing twist, the relative phases among its four wings, its wing-beat amplitude and other flight parameters (Nachtigall, 1989) will affect the wind signal monitored by the rear animal, and thus could also affect the phase it adopts. These factors should also vary the lift experienced by the rear animal.

In some cases, it appeared that the front locust coupled its wing-beat to that of the rear locust. In ten out of 61 coupling sequences, the wing-beat frequency of the front individual alone was adjusted before coupling, and in a further ten sequences adjustments were made by both locusts. However, both locusts continuously make highly erratic changes in wing-beat frequency within approximately ± 1 Hz. If the front locust happens to make an erratic change such that Δf decreases, this could simply increase the probability that the rear locust will couple to it and, hence, that the frequency changes found for the front locust might be entirely random, rather than a response to Δf . Random changes of its wing-beat frequency in the opposite direction also occur, but these would not lead to wing-beat coupling (because Δf would increase). In any case, the incidence of a phase change in the rear locust's wing-beat only ($N=41$) was far greater than that in the front locust's wing-beat only ($N=10$).

As mentioned previously, the coupling strength should also determine the phase values at which coupling occurs. It seems likely that the coupling strength decreases with locust separation. Although we do not know exactly which wind parameters are monitored to produce coupling, the most probable is the periodic change in peak wind speed, which is known to decrease at a rate of 2.4 cm s^{-1} for each 1 cm distance behind the front locust (Kutsch *et al.* 1994). In theory (Fig. 1), a lower coupling strength should give a wider range of phases,

and two observations are consistent with this prediction. First, the scatter in the data was usually lowest when the locusts were closest (at 7.5 cm; Figs 3, 4). Second, the difference in phase between couplings at $+\Delta f$ or $-\Delta f$ was smaller for the two data sets sampled at a distance of 7.5 cm (0.13 and 0.36) than for most of the data sets sampled at 11 cm (0.47, 0.42, 0.39 and 0.19). Both observations suggest that a weakening of the *Zeitgeber* signal with increasing distance gave rise to greater scatter of phase. However, we cannot eliminate the possibility that this increased scatter was due to an increase in the scatter of some key parameters of the *Zeitgeber* signal with distance.

In biological systems of coupled oscillators, it is rarely possible to vary the coupling strength experimentally. In this case, coupling strength can be varied merely by changing the locust separation; i.e. although the flow field is highly complex, the signal it provides to the rear locust decreases with distance. It is also rare to be able completely to silence an individual biological oscillator, which we can achieve simply by rapidly moving one locust to the side of the wind tunnel using the tethering arm (Fig. 2). Our system therefore offers the possibility of analyzing a real system of several coupled oscillators under conditions favourable for experimental manipulation.

The locusts in this study were positioned quite close together, up to a maximum of 15 cm (except in Fig. 10) head-to-head, or approximately 10 cm tail-to-head. Even under these conditions, coupling sequences rarely continued for very long, with an overall mean of 17% of the total flight time accounted for by the 239 criterion coupling sequences (Figs 3, 4). Given this low incidence of coupling, one may question whether it is biologically meaningful. There are several reasons to believe that this coupling is, in locusts, a specific adaptation for flying in groups. First, locusts apparently do fly close together in crowded swarms, with separations as low as 10 cm perhaps even being common under these conditions (Gunn *et al.* 1948;

Rainey, 1976, 1989; Baker *et al.* 1984). Second, it is already known that wing-beat coupling occurs at distances up to at least 30 cm (Kutsch *et al.* 1994). Third, our value of 17% is likely to underestimate the total time spent in criterion coupling, as mentioned above. Moreover, aside from criterion coupling, a substantial percentage of the time consisted of briefer coupling intervals or coupling tendencies. For example, in Fig. 6, following a long coupling sequence at the start of the record, although further criterion coupling did not occur, 64% of the following wing-beats occurred at phases between 0.5 and 1. In total, therefore, it would appear that for at least 85% of the time the two locusts would be flying within this range of phases.

The fourth reason to suggest that wing-beat coupling is a specific evolutionary adaptation to swarm flight is that tethered locusts in the laboratory are known to fly more sluggishly than in nature (Krüppel and Gewecke, 1985; Zarnack and Wortmann, 1989; Robertson and Johnson, 1993). The measured lift production for the tethered locusts flying alone in this study was typically approximately 50% of the locust's mass (Fig. 10). An untethered locust flying in this way, of course, would fall. In producing approximately twice the lift, and thus approximately twice the total force output during flight, a freely flying locust should exert a much stronger effect on those flying behind, perhaps giving rise to a higher incidence of coupling, occurring over a greater distance. Also, the locusts used in this study have been captive-bred for many generations; those used in Jerusalem have been in culture since being collected in Africa in the 1930s. Over these 60 years, there have been around 500 generations, kept living in small cages with no opportunity for flight. The fact that strong flight and wing-beat coupling have persisted suggests that this behaviour is firmly entrenched genetically in this species.

It is noteworthy that wing-beat coupling occurs, at any given distance, specifically at phases associated with an increase in lift (Fig. 9). Thus, this coupling contributes positively to lift, which is the greatest energy demand on a flying locust.

It should be noted that we do not yet know what specific energetic benefit the rear locust obtains from the increased lift that it receives when flying at particular phases (either coupled at that phase or drifting through it). It is possible that, during such periods, the rear locust might reduce its own energy output and yet remain at the same elevation, thus permitting net energy savings. A second possibility is that the increased lift would raise it out of the flow field of its immediate front neighbour, taking it into a location of lower air turbulence. Third, it is possible that the measured lift simply compensates for the downward force produced by the wake of the front locust, thus allowing the rear locust to fly unaffected through the turbulence created by its neighbours.

We have recently observed that, during periods of coupling, there are also changes in the relative phases among various flight muscles of the rear animal and in the number of spikes per wing-beat in some of these muscles (J. M. Camhi and G. Wendler, in preparation). This suggests that the increase in lift may be, in part, an active process.

The nature of the coupling signal is, as yet, unknown. However, whether it is rhythmic changes in wind speed, direction, turbulence or some combination of these, this signal must be detected above that already present from the locust's own wing-beat (Horsmann *et al.* 1983). This presumably complex interaction between these two signals is currently being studied by flow analysis techniques.

Finally, it should be recalled that a real swarm consists of more than just two locusts. In a very dense swarm, a given individual may experience the flow field of several neighbours simultaneously (Kutsch *et al.* 1994). This could create highly complex situations regarding both sensory detection of multiple rhythms and coupling to these nearby wing-beats.

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References

- ASCHOFF, J. (1963). Comparative physiology: Diurnal rhythms. *A. Rev. Physiol.* **25**, 581–600.
- BAKER, P. S., GEWECKE, M. AND COOTER, R. J. (1984). Flight orientation of swarming *Locusta migratoria* L. III. Wing-beat frequency, flight speed and attitude. *Physiol. Ent.* **9**, 247–252.
- BRODSKY, A. K. (1991). Vortex formation in the tethered flight of the peacock butterfly *Inachis io* L. (Lepidoptera, Nymphalidae) and some aspects of insect flight evolution. *J. exp. Biol.* **161**, 77–95.
- COOTER, R. J. (1989). Swarm flight behavior in flies and locusts. In *Insect Flight* (ed. G. J. Goldsworthy and C. H. Wheeler), pp. 165–203. Boca Raton, FL: CRC Press.
- DOMBROWSKI, U. (1991). Untersuchungen zur funktionellen Organisation des Flugsystems von *Manduca sexta* L. Dissertation, Universität zu Köln.
- GUNN, D. L., PERRY, F. C., SEYMOUR, W. G., TELFORD, T. M., WRIGHT, E. N. AND YEO, D. (1948). Behavior of the desert locust in relation to aircraft spraying. *Anti-locust Bull.* **3**, 1–70.
- HORSMANN, U. Y., HEINZEL, H.-G. AND WENDLER, G. (1983). The phasic influence of self-generated air current modulations on the locust flight motor. *J. comp. Physiol.* **150**, 427–438.
- JENSEN, M. (1956). Biology and physics of locust flight. III. The aerodynamics of locust flight. *Phil. Trans. R. Soc. Lond. B* **239**, 511–552.
- JOHNSON, J. (1969). *Migration and Dispersal of Insects by Flight*. London: Methuen.
- KRÜPPEL, T. AND GEWECKE, M. (1985). Visually induced flight maneuvers in the tethered locust (*Schistocerca gregaria*). In *Insect Locomotion* (ed. M. Gewecke and G. Wendler), pp. 167–174. Berlin: Paul Parey.
- KUTSCH, W., CAMHI, J. AND SUMBRE, G. (1994). Close encounters among flying locusts produce wing-beat coupling. *J. comp. Physiol.* **174**, 643–649.
- MÖHL, B. (1989). Sense organs and the control of flight. In *Insect Flight* (ed. G. J. Goldsworthy and C. H. Wheeler), pp. 75–98. Boca Raton, FL: CRC Press.

- NACHTIGALL, W. (1989). Mechanics and aerodynamics of flight. In *Insect Flight* (ed. G. J. Goldsworthy and C. H. Wheeler), pp. 1–30. Boca Raton, FL: CRC Press.
- RAINEY, R. C. (1976). Flight behavior and features of the atmospheric environment. In *Insect Flight* (ed. R. C. Rainey), pp. 75–112. Oxford: Blackwell.
- RAINEY, R. C. (1989). *Migration and Meteorology*. Oxford: Clarendon Press.
- ROBERTSON, R. M. AND JOHNSON, A. G. (1993). Collision avoidance of flying locusts: Steering torques and behavior. *J. exp. Biol.* **183**, 35–60.
- SNODGRASS, R. E. (1929). The thoracic mechanism of a grasshopper and its antecedents. *Smiths. misc. Collns* **82**, 1–111.
- VON HOLST, E. (1939). Relative coordination as a phenomenon and as a method of analysis of central nervous function. Translated into English. In *Behavioral Physiology of Animals and Man: The Collected Papers of Erich von Holst* (1973), pp. 33–135. Coral Gables, FL: University of Miami Press.
- WENDLER, G. (1974). The influence of proprioceptive feedback on locust flight co-ordination. *J. comp. Physiol.* **88**, 173–200.
- WENDLER, G. (1983). The interaction of peripheral and central components in insect locomotion. In *Neuroethology and Behavioral Physiology* (ed. F. Huber and H. Markl), pp. 42–53. Heidelberg: Springer.
- WILSON, D. M. (1967). An approach to the problem of control of rhythmic behaviors. In *Invertebrate Nervous Systems* (ed. C. A. G. Wiersma), pp. 219–230. Chicago: University of Chicago Press.
- ZAR, J. (1974). *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall.
- ZARNACK, W. AND WORTMANN, M. (1989). On the so-called constant-lift reaction of migratory locusts. *J. exp. Biol.* **147**, 111–124.