ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Does metabolic rate at rest and during flight scale with body mass in insects?

by

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Electronic appendix A – Methods, Raw Data and Statistics

Several studies have reported that insect RMR may vary between insects that fly and those that do not (Full, 1997; Reinhold 1999; Addo-Bediako *et al*. 2002). Therefore, we restricted our analysis to insect orders in which individual species had been measured both during flight and at rest using similar methods. Values for metabolic rates (mm^3 O_2 consumption h^{-1}) or mass-specific metabolic rates (mm^3 O_2 consumption mg^{-1} h^{-1}) and body mass (mg) were compiled from the available literature. The measurements of both RMR and FMR were adjusted to 22ºC assuming a Q10 of 2.0 for both resting and flight metabolism (Morgan *et al*. 1985). For a few species, several studies had measured the MR, we took the mean across these studies to prevent these species biasing our analyses. Mass-specific MRs (sRMR and sFMR) were calculated by dividing MR by wet body mass (*M*).

We normalised all data by logarithmic ($\log_{10}$) transformation and fitted functional relationships of the form $\log_{10}(Y) = \log_{10}(a) + b\log_{10}(M)$, where Y is RMR, sRMR, FMR or sFMR. Since both variables (MR and *M*) were subject to error and *M* was not under the control of the investigators, Model II regression techniques such as reduced major axis regression (RMA) should be employed (Sokal and Rohlf 1995).

We wanted to investigate whether there was a difference in the functional relationship between FMR or sFMR and body mass for small (<10mg) and large (>10mg) insects using analysis of covariance (ANCOVA). However, Model II regression techniques have not been developed in ANCOVA (Sokal and Rohlf 1995), therefore, we employed least-squares regression techniques throughout. Further, RMA produced similar scaling relationships to those obtained using least-squares regression (table 3; figure 1). Using ANCOVA we compared three models; (1) allowing for different exponents and intercepts for small and large insects (including interaction of size), (2) fitting the same exponent for both small and large insects, but allowing for different intercepts (additive effect of size) and (3) fitting one intercept and slope for both small and large insects (least-squares regression). We used *t*-tests to determine whether the regression coefficient (*b*) deviated significantly from previously suggested scaling relationships (Sokal and Rohlf 1995).

Temperature Correction

The resting metabolic rates (RMR) and mass-specific RMR of insects were normalized to 22ºC, using the Q10 correction for temperature differences. Q10 is the factorial increase in biological process, in this case RMR, with a temperature change of 10ºC. The Q10 value of 2 was used because there is empirical evidence that this value is applicable to insects (Morgan *et al*. 1985). Data were transformed using the following equation:

$$RMR_N = RMR_E \cdot 10^{(T_N - T_E)\log(Q10)/10}$$

Where RMR_N is the RMR at the normalized temperature, RMR_E is the RMR measured at the experimental temperature, T_N is the temperature to which the RMR is normalized and T_E is the experimental temperature.

Table 1. Body mass, temperature at which measurements were performed, and temperature normalized resting metabolic rate (RMR) and mass-specific RMR (sRMR) of insect species from the literature.

Order	Species	Mass (mg)	Temp. (ºC)	RMR (mm^3 O_2 h^{-1})	sRMR (mm^3 O_2 mg^{-1} h^{-1})	Reference
Dictyoptera	*Periplaneta americana*	990.5	25	300.86	0.36	Poláček and Kubišta (1960)
	Blattella germanica	51	28.5	24.68	0.48	Coelho and Moore (1989)
	Blatta orientalis	331	26	90.46	0.27	
	Nauphoeta cinerea	515	22.7	106.46	0.21	
	Eublaberus posticus	2200	25	607.56	0.28	
	Leucophaea maderae	2800	20	418.13	0.15	
	Gromphodorina chopardi	3400	25	386.63	0.11	
	Blaberus discoidalis	4080	25	563.38	0.14	
	Byrsotria fumagata	4950	25	804.13	0.16	
	Gromphodorina portentosa	5200	24	860.10	0.17	
	Blaberus giganteus	5165	23	1278.93	0.25	
Diptera	*Pantophthalmus tabaninus*	1764	25	472.83	0.27	Bartholomew and Lighton (1986)
	Aedes campestris	6.72	17.5	41.90	6.23	Hocking (1953)
	Drosophila repleta	3.28	25	13.32	1.67	Chadwick and Gilmour (1940)
	D. americana	1.1	20	1.49	1.36	Chadwick (1947)
	D. virilis[§]	1.47	20	2.60	1.77	Chadwick (1947)/ Lehmann *et al*. (2000)[†]
	D. melanogaster[§]	0.93	22	3.70	5.0	Hocking (1953)/ Lehmann *et al*. (2000)[†]
	D. nikananu	0.49	22	1.65	3.36	Lehmann *et al*. (2000)[†]
	D. mimica	2.44	22	5.61	2.3	
	Tabanus affinis	160	18.9	158.68	0.99	Hocking (1953)
	Simulium venustum	2.5	20.6	12.12	4.85	
	Nowickia nitida/rostrata	130.4	30	185.74	1.42	Chappell and Morgan (1987)
	Glaphyropygia dryas	20.0	24	36.21	1.81	Morgan *et al*. (1985)
	Promachus	200.2	24	202.17	1.01	
	Musca domestica	18	25	52.63	2.92	Fullmer and Hoskins (1951)*
	Phormia regina	37.5	25	96.86	2.58	Keister and Buck (1961) /Buck and Keister (1949)
Hemiptera	*Cystosoma saundersii*	940	25	458.11	0.49	Morgan (1987)
	Fidicina mannifera	2838	22	1560.9	0.55	Bartholomew and Barnhart (1984)
Hymenoptera	*Apis mellifera*[§]	101.7	22.7	326.14	3.21	Jongbloed and Wiersama (1934)/ Hocking (1953)/ Rothe

						and Nachtigall (1989)
Lepidoptera	*Mimas tiliae*	299.4	22.3	219.9	0.73	Zebe (1954)
	Antheraea pernyi	1064.4	22.2	766.31	0.72	
	Odonestis pruni	270	21.3	175.72	0.65	
	Vanessa io	232.5	21.4	133.31	0.57	
	Metopsilus porcellus	285	21.4	196.09	0.69	
	Bombyx mori	720	23	698.66	0.97	Itaya (1940)*
	Galleria mellonella	65	30	85.87	1.32	
	Manduca corallina	1618.25	23	498.26	0.31	Bartholomew and Casey (1978)
	Perigonia lusca	547.25	23	188.92	0.36	
	Enyo ocypete	411	23	176.40	0.43	
	Xylophanes pluto	828.00	23	285.84	0.35	
	Automeris jacunda	653.25	23	237.71	0.36	
	Automeris zugana	488.43	23	168.62	0.35	
	Eacles imperialis	1105	23	556.74	0.50	
	Syssphinx molina	1757	23	1196.72	0.68	
	Adeloneivaia boisduvalii	995.33	23	995.33	0.28	
Odonata	*Anax junius*	1019	30	596.97	0.59	May (1979)
	Brachymesia simpliciollis	344	30	223.26	0.65	
	Erythemis simplicicollis	263	30	126.89	0.48	
	Erythrodiplax berenice	125	30	63.18	0.51	
	Erythrodiplax connata	52	30	37.93	0.72	
	Libellula auripennis	464	30	199.87	0.43	
	Miathyria marcella	171	30	149.28	0.87	
	Pachydiplax longipennis	186	30	101.49	0.54	
	Pantala flavescens	339	30	241.43	0.71	
	Perithemis tenera	61	30	47.65	0.78	
	Tramea carolina	383	30	257.37	0.67	
Orthoptera	*Schistocerca gegaria*§	1736.45	26.4	757.45	0.44	Bodenheimer (1929)/ Krogh and Weis-Fogh (1951)
	Neoconcephalus robustus	870	23	616.92	0.71	Stevens and Josephson (1977)
	Euconcephalus nastus	650	23	169.81	0.26	
	Gryllotalpa australis	874	23	342.50	0.39	Kavanagh (1987)
	Teleogryllus commodus	602	23	174.12	0.29	

*denotes a reference and data that was obtained from Keister and Buck (1974).

†Measurements of CO_2 production at rest or during flight were converted to O_2 consumption assuming a respiratory quotient of 1.

§ Measurements for the same species from several studies were averaged.

Table 2. Body mass, temperature at which measurements were performed, and temperature normalized flight metabolic rate (FMR) and mass-specific FMR (sFMR) of insects from the literature.

Order	Species	Mass (mg)	Temp. (ºC)	FMR (mm^3 O_2 h^{-1})	sFMR (mm^3 O_2 mg^{-1} h^{-1})	Reference
Dictyoptera	*Periplaneta americana*	1.42	20	53828	37.91	Poláček and Kubišta (1960)
Diptera	*Aedes flavescens*	3.15	15.56	107.80	34.22	Hocking (1953)
	Aedes nearticus	5.77	15	193.09	33.46	
	Drosophila americana	1.25	20	34.16	27.33	Chadwick (1947)
	D. virilis[§]	1.35	20	22.52	36.59	Chadwick (1947)/ Lehmann *et al*. (2000)[†]
	D. repleta	3.28	25	56.11	17.11	Chadwick and Gilmour (1940)
	D. melanogaster[§]	0.9	25	28.45	27.88	Hocking (1953)/ Lehmann *et al*. (2000)[†]
	D. nikananu	0.64	22	20.90	32.65	Lehmann *et al*. (2000)[†]
	D. mimica	3.07	22	119.21	38.83	
	Lucilia sericata	32.55	27	2279.08	70.02	Davis and Fraenkel (1940)
	Nowickia nitida/rostrata	130.4	30	3984.421	30.56	Chappell and Morgan (1987)
	Tabanus affinis	160	18.9	4363.77	27.27	Hocking (1953)
	Simulium venustum	2.5	20.6	74.38	29.75	
	Glaphyropygia dryas	20.0	24	795.51	39.78	Morgan *et al*. (1985)
	Promachus	200.2	24	9233.58	46.12	
Homoptera	*Fidicina mannifera*	2838	22	108922.4	38.38	Bartholomew and Barnhart (1984)
Hymenoptera	*Bombus lucorum*	511.33	32	15552.1	30.42	Ellington *et al*. (1990)
	Euglossa dissimula	104	22	13000	125	Casey *et al*. (1985)
	Euglossa mandibularis	90	22	17460	194	
	Euglossa imperialis	170	22	20699.2	121.76	
	Euglossa saphirina	71	22	8500.12	119.72	
	Exaerete frontalis	640	22	44832	70.05	
	Eufriesia pulchra	430	22	41499.3	96.51	
	Eulaema nigrita	400	22	53352	133.38	
	E. cingulata	550	22	50077.5	91.05	
	E. meriana	940	22	60996.6	64.89	
	Apis mellifera[§]	94.16	21.73	8259.07	87.72	Withers (1981)/ Jongbloed and Wiersama (1934)/ Hocking (1953)
	Bombus edwardsii	400	22	31200	78	Heinrich (1975)
	Xylocopa californica	600	22	37800	63	Chappell (1982)

	Xylocopa capensis	1200	22	63600	53	Nicholson and Louw (1982)
Lepidoptera	*Hyles euphorbia*	650	22	35945	55.3	Heinrich and Casey (1973)
	Deilephila elepenor	650	22	38610	59.4	
	Mimas tiliae	291	22.56	16237.8	55.8	Zebe (1954)
	Antheraea pernyi	595	22.6	16780.41	28.20	
	Odonestis pruni	240	20.9	16577.13	69.07	
	Vanessa io	176.25	21.625	10014.9	54.73	
	V. polychloros	270	22.7	18649.27	69.07	
	Metopsilus porcellus	243.75	22.55	21026.8	86.53	
	Deilephila euphorbiae	395	22.3	24759.75	62.68	
	Aglia tau	112.5	21.9	7861.81	69.88	
	Saturnia pavonia	198.33	21.43	16850.38	84.96	
	Cucullia lactucae	285	22.6	7873.64	27.63	
	Plusia gamma	120	23.4	4622.90	38.52	
	Agrotis exclaationis	200	23.7	8248.46	41.24	
	A. pronuba	273.33	22.07	11769.74	43.06	
	Manduca corallina	1618.25	23		64.78	Bartholomew and Casey (1978)
	Protambulyx strigilis	1095.33	23	62299.84	56.88	
	Perigonia lusca	547.25	23	25310.56	46.25	
	Enyo ocypete	411	23	34957.72	85.06	
	Xylophanes pluto	828.00	23	51204.7	61.84	
	Automeris jacunda	653.25	23	26074.57	39.92	
	Automeris zugana	488.43	23	25101.13	51.39	
	Eacles imperialis	1105	23	50116.98	45.35	
	Syssphinx molina	1757	23	105507.9	60.05	
	Adeloneivaia boisduvalii	995.33	23	35809.74	35.98	
Orthoptera	*Schistocerca gregaria*	1960	27.6	21404.5	10.92	Krogh and Weis-Fogh (1951)

†Measurements of CO_2 production at rest or during flight were converted to O_2 consumption assuming a respiratory quotient of 1.

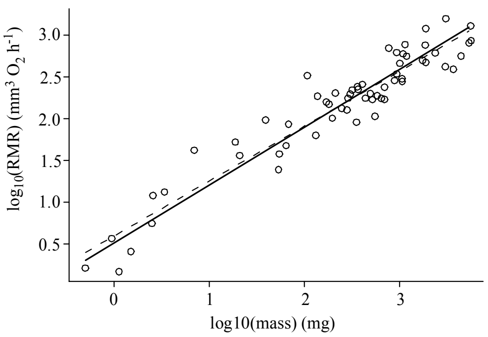
§ Measurements for the same species from several studies were averaged.

Table 3. Scaling relationships of resting (RMR) and flight metabolic rate (FMR). Slopes and intercepts ± s.e. are given for reduced major axis regression (RMA) and ordinary least-squares regression. Analyses of Covariance (ANCOVA) for FMR allowing for separate intercepts for small (<10 mg; upper intercept) and large (>10mg; lower) insects are given in the last column.

	RMA		Least Squares		ANCOVA	
	Slope	Intercept†	Slope	Intercept†	Slope	Intercept†
RMR	0.69±0.03	0.54±0.07	0.66±0.03	0.62±0.07		
sRMR	-0.41±0.03	0.76±0.07	-0.34±0.03	0.62±0.07		
FMR	1.01±0.03	1.49±0.08	1.07±0.03	1.55±0.08	0.87±0.06	2.11±0.18
						1.50±0.18
sFMR	0.25±0.03	1.16±0.08	0.07±0.03	1.55±0.08	-0.14±0.06	2.11±0.18
						1.50±0.18

† Values represent log_{10}(Intercept).

(a)



(b)

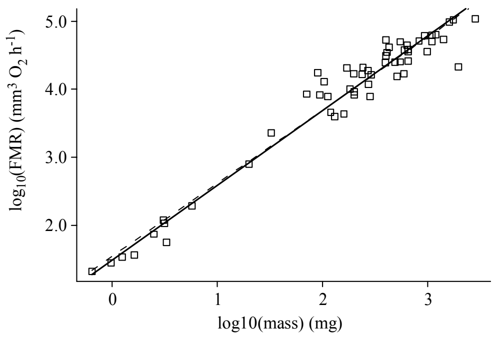


Figure 1. Comparison of the relationship between body mass and metabolic rate obtained using reduced major axis (solid line) or least-squares linear (dashed) regression analyses for (*a*) RMR and (*b*) FMR.

Electronic appendix B - Effect of tethering

The FMR of several of the insects were determined during tethered flight, which, if flight force was not monitored to ensure it was the same as during free flight, may reduce the observed FMR and account for the difference between small and large insects. To ensure that tethering did not influence our results, we used estimates of the reduction in MR during tethered flight to correct the observed FMR. Direct experimental comparisons during tethered and free flight suggest that tethering may reduce sFMR by 20-50% (Bartholomew and Barnhart 1984; Morgan *et al*. 1985). We used the larger of these estimates (50%) to correct the FMR of tethered insects. As with the uncorrected data, FMR increased with increasing body mass after correcting for the effect of tethering (FMR = 50.49 $M^{1.02}$; $F_{1,54} = 1284$; $p < 0.0001$). Partitioning of the data into two clusters (>10mg or <10mg) was best fitted by two power law regressions with the same exponents ($F_{1,52} = 0.44$; p = 0.51), but significantly different intercepts ($F_{1,53} = 8.37$; $p = 0.0056$) (figure 1). After correction for tethering, the power law regression for large insects was FMR = 124.96 $M^{0.87}$, whereas the power law regression for small insects was FMR = 46.97 $M^{0.87}$ (figure 1). This suggests that tethering is not sufficient to explain the extremely low sFMR in small insects.

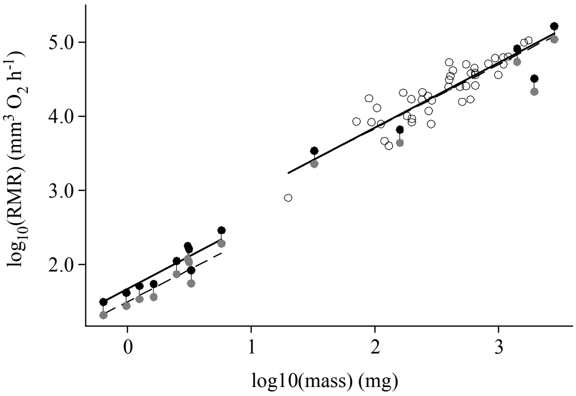


Figure 1. The effect of tethering upon the relationship between insect body mass and FMR. Species recorded in free flight (open circles) and tethered (grey circles), black circles show tethered species adjusted for the possible effects of tethering by 50%. The dashed line indicates the original least-squares relationship; the solid line the relationship after adjustment for tethering.

References

Bartholomew, G.A., Barnhardt, M.C. 1984 Tracheal gases, respiratory gas exchange, body temperature and flight in some tropical cicadas. *J. Exp. Biol.* **111**, 131-144.

Bartholomew, G.A., Casey, T. 1978 Oxygen consumption of moths during rest, pre-flight warm-up, and flight in relation to body size and wing morphology. *J. Exp. Biol*. **76**, 11-25.

Bartholomew, G.A., Lighton, J.R.B. 1986 Endothermy and energy metabolism of a giant tropical fly, *Pantophthalmus tabaninus* Thunberg. *J. Comp. Physiol. B* **156**, 461-467.

Buck, J., Keister, M. 1949 Respiration and water loss in the adult blowfly, *Phormia regina*, and their relation to the physiological action of DDT. *Biol. Bull*. **97**, 64-81.

Casey, T.M., May, M.L., Morgan, K.R. 1985 Flight energetics of euglossine bees in relation to morphology and wing stroke frequency. *J. Exp. Biol*. **116**, 271-289.

Chadwick, L.E. 1947 The respiratory quotient of *Drosophila* in flight. *Biol. Bull*. **93**, 229-239.

Chadwick, L.E., Gilmour, D. 1940 Respiration during flight in *Drosophila repleta* Wollaston: the oxygen consumption considered in relation to wing rate. *Physiol. Zool*. **18**, 398-410.

Chappell, M.A. 1982 Temperature regulation of carpenter bees (*Xylocopa californica*) foraging in the Colorado Desert of Southern California. *Physiol. Zool*. **55**, 267-80.

Chappell, M.A., Morgan, K.R. 1987 Temperature regulation, endothermy, resting metabolism, and flight energetics of tachinid flies (*Nowickia* sp.). *Physiol. Zool*. **60**, 550-559.

Coelho, J.R., Moore, A.J. 1989 Allometry of resting metabolic rate in cockroaches. *Comp. Biochem. Physiol. A* **94**, 587-590.

Davis, R.A., Fraenkel, G. 1940 The oxygen consumption of flies during flight. *J. Exp. Biol.* **17**,402-407.

Ellington, C.P., Machin, K.E., Casey, T.M. 1990 Oxygen consumption of bumblebees in forward flight. *Nature* **347**, 472-473.

Full, R.J. 1997 Invertebrate locomotor systems. In: *The handbook of comparative physiology*. Ed. Dantzler, W., Oxford, OUP.

Heinrich, B. 1975 Thermoregulation in bumblebees II. Energetics of warm-up and free flight. *J. Comp. Physiol*. **96**, 155-166.

Heinrich, B., Casey, T.M. 1973 Metabolic rate and endothermy in Sphinx Moths. *J. Comp. Physiol*. **82**, 195-206.

Hocking, B. 1953 The intrinsic range and speed of flight of insects. *Trans. Roy. Ent. Soc. (Lond.)* **104**, 223-345.

Jongbloed, J., Wiersma, C.A.G. 1934 Der Stoffwechsel der Honigbiene während des Fliegens. *Z. Vergl. Physiol*. **21**,519-533.

Kavanagh, M.W. 1987 The efficiency of sound production in two cricket species, *Gryllotalpa australis* and *Teleogryllus commodus* (Orthoptera, Grylloidea). *J. Exp. Biol*. **130**, 107-119.

Keister, M., Buck, J. 1961 Respiration of *Phormia regina* in relation to temperature and oxygen. *J. Insect Physiol*. **7**, 51-72.

Keister, M., Buck, J. 1974 Respiration: some exogenous and endogenous effects on rate of respiration. In *The Physiology of Insecta,* vol. 6 (ed. M. Rockstein), pp.469-509 London: Academic Press.

Krogh, A., Weis-Fogh, T. 1951 The respiratory exchange of the desert locust before, after and during flight. *J. Exp. Biol*. **28**, 344-357.

Lehmann, F.–O., Dickinson, M.H., Staunton, J. 2000 The scaling of carbon dioxide release and respiratory water loss in flying fruit flies (*Drosophila* spp.). *J. Exp. Biol*. **203**, 1613-1624.

May, M.L. 1979 Energy metabolism of dragonflies (Odonata: anisoptera) at rest and during endothermic warm up. *J. Exp. Biol*. **83**, 79-94.

Morgan, K.R. 1987 Temperature regulation, energy metabolism and mate searching in rain beetles (*Plecoma* spp.), winter active, endothermic scarabs (Coleoptera). *J. Exp. Biol.* **128**,107-122.

Morgan, K.R., Shelly, T.E., Kimsey, L.S. 1985 Body temperature regulation, energy metabolism, and foraging in light-seeking and shade-seeking robber flies. *J. Comp. Physiol. B* **155**, 561-570.

Nicholson, S.W., Louw, G. 1982 Simultaneous measurement of evaporative water loss, oxygen consumption and thoracic temperature during flight in a carpenter bee. *J. Exp. Zool.* **222**, 287-96.

Poláček and Kubišta 1960 Metabolism of the cockroach *Periplaneta americana* during flight. *Physiol. Bohemoslov.* **9**, 228-234.

Rothe, U., Nachtigall, W. (1989) Flight of the honey bee IV. Respiratory quotients and metabolic rates during sitting, walking and flying. *J. Comp. Physiol. B* **158**, 739-749.

Sokal, R.R., Rohlf, F.J. 1995 *Biometry: The principles and practice of statistics in biological research.* Third edition. New York: W.H. Freeman and Company.

Stevens, E.D., Josephson, R.K. 1977 Metabolic rate and body temperature in singing katydids. *Physiol. Zool*. **50**, 31-42.

Withers, P.C. 1981 The effects of ambient air pressure on oxygen consumption of resting and hovering honeybees. *J. Comp. Physiol. B* **141**, 433-437.

Zebe, E. 1954 Über den Stoffwechsel der Lepidopteren. *Z. Vergl. Physiol*. **36**, 290-317.