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Oxygen consumption and the evolution of order: negentropy criteria applied to the evolution of ants

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Abstract. Optimization of energy use by evolving organisms, predicted by theoretical extensions of the neo-Darwinian theory, is contrasted with that of irreversible thermodynamics, which predicts an increase in orderliness and thus an increase in energy consumption per unit of biomass. We compared this index with estimates of social complexity among ant genera and species. Our results show that simple optimization models cannot explain experimental data, and that social complexity correlates differently with negentropy at different levels of analysis. Comparing the genera among Formicidae, workers (not colonies) from genera with highly social species are less negentropic than those of socially primitive ones. At the sub-generic level, social complexity correlated positively with negentropy among species, for major workers in *Acromyrmex* and for minor workers in *Atta*. The results illustrate the complexity of thermodynamic criteria in the study of evolution but also hint at their usefulness. In this case, they show that two different evolutionary routes to the complex Attini ant societies may exist.
Key words. Negentropy; oxygen consumption; social evolution; ants; energy; self-organization; dissipative systems.

Several attempts to base the biological principle of evolution on physical laws include assumptions that natural selection has led to the optimization of living beings according to various criteria, such as average fitness[1], maximal efficiency in resource utilization[2], minimal metabolized energy per unit of biomass[3], maximum energetic power[4], minimal rate of entropy dissipation[5], etc. All these criteria assume that Darwin's principle translates into optimal regimes of operation along metabolic pathways in a biological system. This implies that evolution tends to make energy use by organisms more efficient and thus, all else being equal, energy consumption per unit mass should be reduced over evolutionary time.

An opposing view derives from the approach put forward by the analysis of irreversible thermodynamics in self-organizing systems[5], which assumes an increase in order and thus in energy consumption per unit mass as a thermodynamic necessity of evolution[6]. Nicolis and Prigogine[5] suggest that contact between irreversible thermodynamics and Darwin's idea of the survival of the fittest can probably be made because a low rate of dissipation is likely to give an organism a selective advantage. Lamprecht and Zotin[6], and especially Zotin et al.[7-9], work out the idea more carefully and conclude that the probability state for living organisms may be associated with the respiration intensity value. Only in the course of biological evolution have organisms with increasingly high levels of orderliness appeared step by step. Consequently, the dissipation function is related to the distance of the organism from equilibrium state. This theoretical contradiction allows for experiments which could eventually solve the problem if quantitative measures of order or of energy consumption are used to compare organisms along a phylogenetic tree.

A quantitative measurement of order in living systems has always been difficult[10]. With the advent of irreversible thermodynamics[5], order can be assessed through estimates of entropy. These assessments are based on the assumption that the higher the order or complexity of an organism is, the lower will be the probability state of the system, the longer the evolutionary time to produce the given state, the farther the thermodynamic system from the stationary state or equilibrium, the higher the value of bond dissipation function, the higher the basal metabolic rate, and the larger the energy consumption per unit mass[6-9, 11].

Zotin and Konoplev[7] proposed a negentropy measurement by means of an orderliness criterion (Cr) based on basal metabolic rates, measured through oxygen consumption (Q_{O_2}) normalized by body weight (W), as an index of order for living organisms; where:

$$Q_{O_2} = a \cdot W^{-b}$$

and

$$Cr = k \cdot a/T$$

where a and b are species-specific constants; a is the oxygen consumption at unit weight and b describes the variation in oxygen consumption due to variation in weight. T is the absolute temperature and $k = 3490\ h \cdot {}^{\circ}K/cal$. This index assumes that heat production in adult animals is equivalent to respiratory intensity[9].

We may define a new orderliness criterion Cr′ for a temperature of 25 °C, so that:

$$Cr'_{25} = a$$

Zotin and Konoplev[7], using data from around 100 different species summed up by Hemmingsen[12], showed that Cr gives values which reflect the phylogenetic history among vertebrates and among invertebrates, if comparisons are made at the level of classes, or at the genus level[8]. One limitation of this work is that it was developed with solitary organisms in mind, and adaptations of the approach are required if we want to apply it to social systems.

In ants, an equivalent approach used by Jaffe[11] measured the degree of order through the information content of the communication system used for recruitment to food. An orderliness criterion (negentropy content of the chemical communication systems) was shown to correlate positively with social complexity, and thus could be used in phylogenetic analysis. Here, we explore the usefulness of orderliness criteria, obtained through metabolism measurements, in assessing social evolution in ants.

Methods

We correlated Cr′ with the social complexity of ants in the subfamilies Formicinae and Myrmicinae, using data from the literature obtained with similar experimental procedures and where oxygen consumption per unit weight (in dry weight) of ant workers at 25 °C is given[13–18]. Social complexity was assessed using a criterion derived from Oster and Wilson[19], based on the number of worker castes. According to this criterion, a value of 1 was given to those genera where all species are monomorphic; a value of 2 if at least one species in the genus is polymorphic, possessing two worker sub-castes; and a value of 3 if at least one species is polymorphic, possessing three worker sub-castes.

We also used data from 8 Attini ants from 4 different genera. *Mycocepurus goeldi*, *Trachymyrmex* sp *a*, *Acronmyrmex subterraneus*, *Acromyrmex coronatus*, *Acromyrmex crassispinus*, *Atta sexdens rubropilosa*, *Atta bisphaerica* and *Atta laevigata* were collected in Minas Gerais and Sao Paulo, Brasil. The measurements of basal oxygen consumption of individual adult worker ants were made using a Warburg respirometer at 25 °C[20,21]. Mass was measured and expressed as fresh weight. Social complexity of these ants was assessed subjectively through field observations estimating colony size and degree of worker size-polymorphism. Castes were assessed through the size of the workers, as Attini have a continuously polymorphic caste system which is strongly related to worker size[19].

Data from Attini species were plotted in two different formats, in order to obtain additional 'orderliness' criteria:

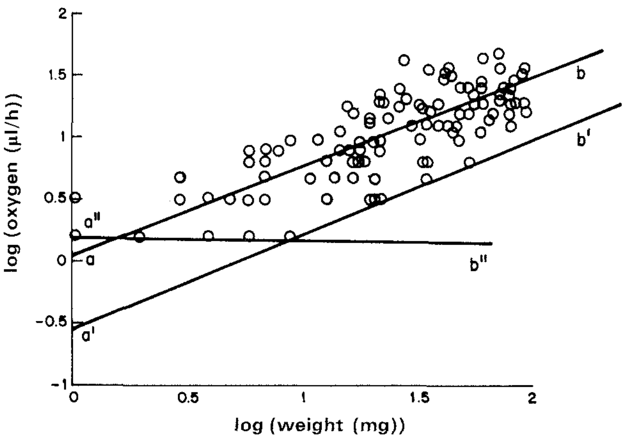

Figure 1. Log-log plot of oxygen consumption per hour vs weight of workers showing the graphically derived orderliness criteria. Data are from *Atta bisphaerica*.

– The log of oxygen consumption at 25 °C expressed in µl/h vs log of fresh weight in mg was used to calculate the values of a and b, applying a linear regression analysis. In addition, these data were plotted (fig. 1), and values of a′, a″, b′ and b″ were found from the graph. These values represent the minimum oxygen consumption per unit weight of the extreme worker forms, i.e. major and minor workers, and the slope of the regression calculated for the metabolic state of minimum oxygen consumption (basal metabolism) for each weight category.

– The log of the quotient of oxygen consumption divided by the fresh weight of the workers vs log of fresh weight of the worker was plotted and the values of slopes c′ and c″ were measured from the graph (fig. 2). The ratio c′/c″ measures the maximum variability in metabolic behavior of the workers of a given species, normalized by body weight.

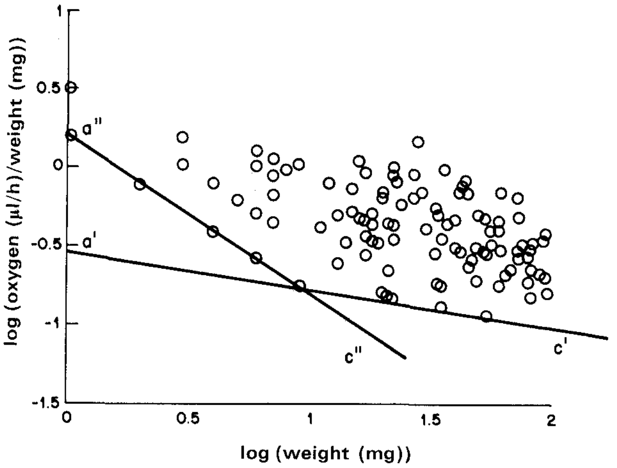

Figure 2. Log-log plot of oxygen consumption rate per unit weight vs weight of individual workers showing the graphically derived orderliness criteria. Data are from *Atta bisphaerica*.

Table 1. Oxygen consumption of individual workers from various paleo- and neoartic ant genera, in relation to the degree of polymorphism of the genus

Genus	n	n_i	µl O_2/mg/h* at 25 °C	Polymorphism number worker castes
Formicinae				
Lasius	4	5	2.23 ± 0.30	1
Formica	6	8	2.14 ± 0.68	2
Camponotus	5	8	2.06 ± 1.17	2
Myrmicinae				
Aphaenogaster	1	1	3.10 ± 0.86	1
Leptothorax	1	1	2.45 ± 0.56	1
Tetramorium	1	1	1.87 ± 0.9	1
Myrmica	2	4	1.52 ± 0.77	1
Novomessor	1	2	1.51 ± 0.99	2
Pogonomyrmex	4	7	1.18 ± 0.46	2

*dry weight of workers in mg.
n, indicates number of species used to calculate the mean for the genus; n_i, indicates the number of data points taken from the literature to calculate the mean; ±, gives the standard deviation taken from the literature (for $n_i = 1$) or derived from the data points. Data taken from tables in Jensen and Nielsen[13], Nielsen, Skyberg and Peakin[17], Maldague, Hardy & Demers[14], Peakin and Josens[18], and Nielsen[15]. Worker caste number from table 1.1 in Oster and Wilson[19].

Results and discussion

Table 1 shows the relation between oxygen consumption per mg of fresh weight of workers per hour and the social complexity of the society, assessed through the number of worker castes. Comparisons were made at the genus level using species of equivalent ecological status. The data show that adult workers of genera with species which have more than one worker caste have a lower basal metabolism, among non-tropical Myrmicinae and Formicinae. If the same analysis is performed among genera of one neotropical ant tribe (table 2), the Attini, this tendency is maintained. The more complex the social structure of the species, the lower the mean basal metabolism of the average workers.

The data in figure 1 show an example of the complex pattern of the distribution of energy consumption among individuals of an ant colony. In all species examined, the energy requirement per unit mass of individuals of an ant colony was related to worker size. As worker size is strongly correlated with the worker caste system in the higher Attini[19], we may suggest that the worker caste system of these societies is reflected in the pattern of oxygen consumption of the workers.

Variations in energy consumption may be due to differences in activity, size and weight of the individuals, but an adjustment to a linear regression using all the data, as done for estimates with solitary organisms, seems inappropriate in this case. The data in figure 1 clearly show at least two lower boundaries in oxygen consumption, which we used to estimate two different linear regressions (a′, a″, b′, b″). The biological meaning of these regressions is thought to be that each species seems to have an energetically optimal worker size (critical sized worker ‘cw’, at the cutting point of the regression b′ with b″). Workers above that size (weight) follow one regression and those below that size another, allowing estimates of at least two castes: minima or minor and maxima or major workers (including soldiers in *Atta*).

At the species level, the degree of polymorphism was assessed through the worker size variability (w max/min: maximum fresh weight recorded/minimum fresh weight recorded) among workers of a single colony of the species, and worker metabolic variability (c′/c″). The two coefficients correlated positively with each other in our data, and both correlated with mean oxygen consumption of workers only if comparisons were limited to the species of one genera (table 3). The same can be said when correlating the value of a with the degree of worker polymorphism. That is, among *Acromyrmex* species, worker polymorphism correlates positively with basal metabolism, as assessed through mean oxygen consumption or through the value of a. In *Atta*, species with higher worker polymorphism showed higher values of a but lower values of mean oxygen consumption (table 3).

A finer analysis of the relation between basal metabolism and polymorphism is shown in table 3. Here we represent the values of a′, a″, b′, b″, c′ and c″. The symbol ′ represents the values derived from a linear regression using large workers, whereas ″ represents those from the smallest workers in the colony, so that

Table 2. Oxygen consumption of individual workers from various Attini ant genera (all neotropical fungus growers) in relation to the degree of social complexity of the species in the genus

Attini genus	n	n_i	µl O_2/mg/h* 25 °C	Colony size** Nr indiv	Degree of work polymorphism
Mycocepurus	1	9	1.7 ± 1.2	10^3	1
Trachymyrmex	1	20	1.6 ± 0.8	10^3	1
Acromyrmex	3	118	1.1 ± 0.3	10^5	2
Atta	3	362	0.6 ± 0.09	10^7	3

*, Fresh weight of workers in mg; **, number of individuals.
O_2 data are from Hebling-Beraldo (unpublished) and from Hebling-Beraldo and Mendes[20, 21]. Data on social structure from personal observations; n, indicates the number of species used to calculate the value for the genus; n_i, indicates the total number of individual workers measured; ±, indicates standard deviation of the mean.

Table 3. Orderliness criteria based on oxygen consumption for various Attini species

	Trachymyrmex	*Mycocepurus goeldi*	*Acromyrmex subterraneus*	*coronatus*	*crassispinus*	*Atta sexdens rubripilosa*	*bisphaerica*	*laevigata*
cw	2.24	0.83	3.98	1.41	1.41	6.31	7.94	19.95
w max/min	1.46	1.75	12.60	15.20	16.50	68.00	99.00	118.00
liv/dry	0.43	0.34	0.44	0.43	0.42	0.37	0.35	0.35
Q_{O_2} (cw)	0.79	0.63	0.28	0.50	0.65	0.32	0.20	0.19
mean Q_{O_2}	1.57	1.72	0.71	1.19	1.26	0.64	0.56	0.47
a	2.50	1.59	0.97	1.22	1.75	1.26	1.37	1.78
b	1.00	1.45	0.79	0.88	0.72	0.72	0.64	0.56
a′	0.78	0.92	0.30	0.59	0.79	0.45	0.30	0.54
a″	1.60	0.56	1.00	0.63	1.05	1.50	1.60	3.10
b′	0.90	1.90	0.68	0.83	0.73	0.75	0.75	0.64
b″	0.00	0.00	0.05	−0.08	−0.08	0.00	0.00	−0.08
c′	−0.01	?	0.00	−0.02	−0.11	−0.15	−0.20	−0.28
c″	?	−1.10	−0.98	−0.98	−0.98	−1.12	−0.99	−0.82
c′/c″	?	?	0.00	0.02	0.11	0.13	0.20	0.34
n	20	9	45	30	43	131	116	115

cw, weight in mg of critical worker size; w, weight of workers; liv/dry, fresh weight/dry weight.
Q, encrgy consumption in ml of O_2/g of fresh weight of ant/h; a, Q of workers weighting 1 mg; b, slope of linear regression between Q and a′, a″, b′, b″, c′, c″: see figures 1 and 2; ? indicate cases were visual adjustment of one of the regressions was questionable; n, number of replicates (adult workers).

the range of values for the various worker sub-castes is covered. Thus, with these methodological improvements, we are able to calculate a, b and c for major workers and for the minima or smallest worker castes. independently.

The results showed that the allometry allowing for an increase in polymorphism differs between *Acromyrmex* and *Atta*. Values of a′ increased with polymorphism in *Acromyrmex* whereas a″ increased with polymorphism in *Atta*. The ratio c′/c″ was continuously correlated with polymorphism among all Attini, suggesting that morphological polymorphism is somehow reflected in a physiological polymorphism. These results indicate that among species of *Acromyrmex*, greater polymorphism is achieved mainly through more complex major workers (which have a greater Cr′), whereas among species of *Atta*, greater polymorphism has as a consequence greater complexity of minima workers. Thus, each genus has different allometric rules for their worker polymorphism.

Conclusions

The dilemma between optimization and increased negentropy content

At first sight, optimization models derived from a broad interpretation of neo-Darwinism seemed to be confirmed by our analysis among ant genera, opposing Zotin and Konoplev's prediction. The 'average' workers from genera with highly social species were found to be less negentropic than those with socially primitive species. A finer analysis shows that the metabolic characteristics are not evenly distributed among individuals of an ant society, making evolutionary predictions based on an 'average' ant worker difficult. Our data showed that social complexity correlates differently with negentropy, depending on the level of analysis. At levels above genera, our results could be interpreted as above, but this analysis does not apply when comparing worker castes among species of a genus, as the thermodynamic prediction holds for Attini if specific worker castes are compared.

In order to explain our findings, we have to separate two different levels of analysis: the evolutionary level, which explains the diversity of species; and the social level, which explains the relations among workers of a colony. Therefore we propose two different complementary hypotheses:

Hypothesis 1. Individuals of highly social ant species are less complex than individuals from simpler ant societies. This is in agreement with previous findings of studies on the complexity of the ant's nervous system[22]. This implies that social complexity may be achieved by simplifying individual constituents, through individual specialization, reaching a more harmonious integration of individuals into the society. Thus, in order to comply with thermodynamic rules, colonies (not individuals) of highly social species have to have higher metabolic rates per unit mass than colonies from less complex ant societies. This can be achieved only if workers of highly complex ant societies are more active (due to increased social interactions) than workers from less complex societies, consuming more energy during a normal social life than workers from socially primitive species. (Higher social complexity among ants is normally achieved through the development of special worker sub-castes with specific morphological adaptations. In the case of 'soldiers', such adaptations include cuticular structures, heavy mandibles, etc., which reduce the mean oxygen consumption rate per unit mass).

Hypothesis 2. Some individuals, or the range of individuals in highly social ant species, are more complex than those from simpler ant societies. This finding is in agreement with previous findings of studies on the complexity of the communication systems used for recruitment by ants[11]. This trend is also predicted by Zotin and Konoplev's orderliness criterion, and seems to occur at least among Attines.

Thus, increase in energy requirements of organisms or social systems, predicted by irreversible thermodynamics, is not contradictory to the fact that evolution may lead to individuals in a colony becoming more simple and thus less exigent in energy demand, as this last adaptation has to do with specialization of parts of a system – the colony; whereas the former one has to do with an evolutionary constraint – an increase in the negentropy content of the system. Thermodynamic rules, in the light of our findings, predict that colonies of highly social species should have higher metabolic rates, i.e. consume more oxygen, compared to those of less complex societies. This may be brought about through increased social interactions, even if individual workers have a lower basal metabolism. The composition of worker castes may also vary. As we showed, different castes (size categories among workers) correlate differently with the negentropy criteria in different genera. The effect of this variation on whole colony oxygen consumption is difficult to assess if the numbers of workers in each worker sub-caste are unknown. Therefore, measurements of oxygen consumption of whole colonies are required to assess whether the evolution towards social behavior is correlated with increased negentropic content of the society.

The generally-accepted assumption that a colony's energy consumption represents the sum of the energy requirements of individual workers[16,23,24] does not seem to hold. Individuals might be less negentropic in more negentropic colonies. In this respect, it is known that ant colonies with complex societies but less negentropic workers consume more energy than those with less complex societies but more negentropic workers[25]. Thermodynamically and ecologically, the colony rather than the individual has to be considered to be the self-sustainable system, i.e. the thermodynamic system, and thus the system to be considered as the self-organizing unit has to be the colony and not the individual (as in solitary species).

Divergent evolutionary routes to complex ant societies

The orderliness criterion applied to worker sub-castes in Attini species showed that if negentropy is measured for specific worker sub-castes, increased social complexity among species (increased complex worker caste polymorhysm measured as increased size difference among workers (see also ref. 19)) correlates with higher negentropy values for some worker sub-castes in a given genus. The trends in energy consumption among worker sub-castes differ between genera, suggesting the existence of genus-specific allometric relations between worker sub-castes. Thus, no uniform evolutionary strategy leading to social complexity seems to exist among ants. The strategies vary, at least between Attini species: *Atta* species have evolved a higher social complexity through more negentropic minima workers, whereas *Acromyrmex* species have done so through their major workers.

Our results indicate the usefulness of respiratory methods to discern between divergent evolutionary strategies, at least as far as worker caste systems are concerned. The fact that each species has a critically-sized worker (at least at the interface between minor and major workers), and that ant colonies have divergent strategies for creating small or larger workers, suggests that each species has evolved more complex worker size polymorphism, starting from a different sized worker prototype for which the biochemical metabolic system was optimized. Some workers, as worker caste systems evolved, became less efficient energetically than the 'cw' energetically optimized worker. The interesting fact is that this increase in energy consumption in minima or maxima workers is discontinuous, i.e. the relation between minimum energy consumption and body weight is discontinuous among workers of a colony, suggesting the presence of complex allometric rules.

Conclusion. Thermodynamic predictions are compatible with our experimental results. In addition, a thermodynamic approach allowed us to discover the existence of at least two different evolutionary routes to social complexity among Attini ants, using various allometric rules to develop worker sub-castes, and giving different metabolic importance to individual workers in the society. This work, although partly inconclusive in discerning between two theoretical evolutionary approaches, allows us to define more concrete predictions in order to solve the dilemma originally stated: in social species, energy consumption of colonies (not individuals) should have a specific evolutionary pattern.

Irreversible thermodynamics, applied to biology, has great heuristic value, and further applications of thermodynamic criteria to phylogenetic problems promise to be fruitful.

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