Acta Protozool. (2007) 46: 325–332

ACTA
PROTOZOOLOGICA

Laboratory Experiments on Testate Amoebae Preservation in Peats: Implications for Palaeoecology and Future Studies

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Summary. Testate amoebae analysis has been shown to be a valuable technique to reconstruct peatland palaeohydrology and thereby past climate change. However there is cause for concern over the potential impact of differential preservation on the record. To investigate the impact of various environmental factors a sequence of *Sphagnum* samples were subjected to treatment with weak acid, nutrient enrichment and desiccation over 28-months and shorter-term experiments with stronger acids. Changes were subtle but statistically significant with three treatments: long-term dessication and short-term acid treatment at two different concentrations. Results suggest that in dry periods the palaeoecological record may be skewed by differential preservation of tests, potentially leading to over-estimation of water table depths.

Key words: Testate amoebae, palaeoecology, transfer functions, preservation, decomposition.

INTRODUCTION

Testate amoebae are unicellular microrganisms (protists) characterised by a hard shell (the test) and found in aquatic and semi-aquatic environments around the world. Tests may be constructed from agglutinated particles (xenosomes), siliceous plates (idiosomes) or protein/calcium secretions and are found preserved in a variety of low-energy sedimentary environments. Tests can be identified to morphospecies on the basis of the test characteristics long after death. This preservation and the closely-defined ecological preferences of many amoebae taxa has led to their widespread use in palaeoecological studies.

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Testate amoebae have been particularly widely used in palaeoecological studies from peatlands where transfer function models have been derived to relate amoebae communities to hydrology and used to produce high-resolution palaeohydrological reconstructions for the Holocene (e.g. Charman and Hendon 2000, Langdon *et al*. 2003, Booth and Jackson 2003, Blundell and Barber 2005). Several observations suggest that there may be problems with testate amoebae preservation which might influence interpretation of the results of such studies:

1. There appear to be distinct differences in amoebae communities between modern and palaeoecological samples (Wilmshurst *et al*. 2003). The most remarked on change is the apparent loss of the genus *Euglypha* in palaeoecological samples. *Euglypha* taxa may comprise a large percentage of the total count in modern

samples (e.g. 28% of total count in samples from northern Greece (Payne and Mitchell 2007)), but *Euglypha* tests are often very rare in palaeoecological samples and may not be found at all below the accrotelm (aerobic zone) of peatlands. In samples from southern Alaska, *Euglypha* taxa comprise over 6% of modern amoebae but have rarely been found below 50 cm and have not been encountered at all below 100 cm (Payne 2005, Payne *et al*. 2006).

2. Many palaeoecological studies have encountered periods where test concentrations are very low and often too low to be countable (Beyens and Chardez 1987, Swindles *et al*. in press, Charman *et al*. 2001).

3. Tests encountered in palaeoecological studies are commonly in a poorer, more degraded condition than those in modern ecological studies. Common features include a higher proportion of deformed and cracked tests, isolated platelets or a reduction in xenosomes on tests (Charman 1999, Patterson and Kumar 2002, Swindles and Roe 2007). The proportion of such degraded tests may increase down the length of a peat core (although this has not been quantified).

4. Chemical treatments such as pollen preparations lead to selective loss of certain taxa and proportionate over-representation of others (Hendon and Charman 1997).

While many of these observations could be explained by other processes, taken overall they suggest both that many tests may be lost from the palaeoecological record and that this may preferentially affect some taxa over others. If differential preservation is widespread then the palaeoecological record may not be a true record of past amoebae communities potentially leading to incorrect palaeoenvironmental inferences.

Test decomposition is undoubtedly a complex process and may be affected by processes as fine-scale as predation of individual amoebae by other micro and macro-fauna (Ogden and Coûteaux 1987). However, a number of suggestions can be made about possible broad environmental controls on test preservation:

1. Acidity. Peatlands are naturally acid systems with a typical pH of around 4 in ombrotrophic bogs. It is possible that low-pH conditions may serve to corrode both the siliceous plates of some taxa and the cement binding the plates in position, leading to decomposition of tests (Swindles and Roe 2007). An opposing observation is that test preservation appears to be poorer in similar environments with higher pH such as fens and saltmarshes; it might reasonably be expected that if test decomposition is biologically mediated it might be less effective in more acidic conditions.

2. Dessication. Wet conditions may serve to limit microbial attack on tests while dry conditions may enhance decomposition (Coûteaux 1992). Test concentrations are often very low in more humified peats representing drier conditions (Charman *et al*. 2001). Wilmshurst *et al.* (2003) showed a correlation between humification and number of amoebae taxa encountered in New Zealand peats. Roe *et al.* (2002) found test concentrations to be very low in the driest samples from a variety of wetland environments. While low test concentration and diversity could simply be because amoebae diversity and abundance is naturally lower in drier sites, ecological studies have not provided good evidence that this is the case.

3. Nutrient status. It has been noted that test concentrations are often low in subsurface samples from more nutrient rich sites such as fens (Tolonen 1986, Jauhiainen 2002, R. Payne unpublished data) and saltmarshes (Roe *et al*. 2002). Peatlands are oligotrophic ecosystems, limited by the supply of major nutrients. Enhanced nutrient supply may encourage microbial action and accelerate the rate of test decomposition.

Based on palaeoecological data it is difficult to untangle preservation issues from real ecological change in the past. One possible alternative approach is by experimentation, a method previously used to investigate the taphonomy of other microfossils (e.g. diatoms: Barker 1992, Barker *et al*. 1994, ostracods: Kontrovitz *et al*. 1998, pollen: Campbell and Campbell 1994). For testate amoebae, Lousier and Parkinson (1981) experimentally tested the decomposition of tests in leaf litter and showed rapid decomposition over only a week, decomposition was particularly rapid for idiosome tests. Swindles and Roe (2007) used experiments to show the susceptibility of peatland amoebae tests to treatment with a strong mineral acid over a short period of time. Taxa such as *Euglypha strigosa* and *Nebela militaris* were shown to be most sensitive to the experimental treatments while taxa such as *Archerella flavum* and *Assulina muscorum* were the most robust. While these results are extremely interesting they are limited by the unrealistic nature of the experimental scenario.

There is a great deal of uncertainty regarding both the pattern of, and environmental controls on, test

preservation. This study attempts to test the impact of the three environmental factors identified above using laboratory experiments and to characterise the potential impact on the palaeoecological record. Specifically, this study attempts to use longer-term, more realistic experimental scenarios than previously tested.

METHODS

Sphagnum samples were collected from 15 locations spanning the hydrological gradient on a mire at Moose Pass in south-central Alaska (Dachnowski-Stokes 1941, Payne *et al*. 2006). Moss samples (50 mm length) were extracted from sub-surface peat (approximately 10–15 cm depth) air-dried and then shredded into pieces of 2–3 mm length. Samples were aggregated to maximise the number of amoebae taxa included by placing the combined samples on a flask-shaker for 24 hours. Four samples were analysed immediately as a control set, a further 24 samples were subjected to experimental treatments. Approximately 2 g of dried *Sphagnum* was placed in a 20 ml glass vial with pierced plastic lid to allow gas exchange. Four samples were randomly assigned to each of four long-term experimental treatments (Table 1). To test the impact of desiccation one set of samples was left air-dried. To test the impact of acidity, one set of samples had a pH 2 solution of H_2SO_4 added. To test the impact of nutrient enrichment, samples were treated with a commonly available horticultural NPK fertiliser ('Baby Bio Time Release') at a lower (0.4 g/sample) and higher (0.8 g/sample) dose. The samples were all stored in the dark at room temperature (c. 20°C) for a period of 28 months and were topped-up with the appropriate fluid to compensate for evaporation at regular intervals. As an additional test, two shorter term experiments were carried out using stronger acids. 20 ml of 4M or 1M H_2SO_4 was added to each of four replicate *Sphagnum* samples for 24 hours. At the end of this period, amoebae samples were prepared for microscopy using standard methods (Hendon and Charman 1997). Sub-samples were removed, boiled in distilled water for approximately 10 minutes and sieved at 250 and 15 µm with the 15 < 250 µm fraction retained. Microscope slides were prepared using glycerol and examined at 400× magnification; a minimum of 150 tests were counted (mean = 158). Amoebae were identified and recorded following the same taxonomic scheme as Payne *et al*. (2006). Amoebae communities were recorded on a percentage basis to maximise relevance to the palaeoecological record. The southern Alaskan transfer function (Payne *et al*. 2006) was used to infer water table depths based on the testate amoebae data (termed 'testate amoebae inferred depth to water table:' TI-DWT). The optimal 2-component WA-PLS model was applied with error estimation by boot-strapping (1000 cycles). Data plotting and transfer function inference was carried out using C[2] ver. 1.4 (Juggins 2003). Shannon-Weiner 'H' was calculated as a simple diversity index. Permutation t-tests (10,000 permutations) were used to test the difference in selected variables between the control and experimental treatments.

Differences between the amoebae communities of the control set and each experimental treatment were tested using a sequence of analyses of similarity (ANOSIM) with a Bray-Curtis distance measure and 10,000 permutations in PAST ver. 1.71 (Hammer *et al*. 2001). ANOSIM is a non-parametric test of similarity between pre-defined groups (Clarke 1993). The test statistic (R_{ANOSIM}) has a value between –1 and +1 (although negative values are unusual). A value of 0 indicates the null hypothesis, that there is no difference between groups while a value of 1 indicates that all samples within groups are more similar to one another than to any samples from different groups.

RESULTS

Testate amoebae communities of the experimental samples are shown in Fig. 1. No live or encysted amoebae were encountered at any point during counting; all changes can therefore be attributed to preservation effects rather than real community changes. No distinct differences in the preservation state of tests was noted between treatments and the control.

Qualitatively, community changes in the treated samples are not particularly distinct (Fig. 1). Only minor differences are obvious between treatments such as higher proportions of *Arcella arenaria* and *Trigonopyxis arcula* in the dried samples and greater abundance of *Heleopera petricola* in the control samples. It is notable that the highest concentration of *Euglypha* tests is in the control sample. This may be important given the apparent sensitivity of the tests of this genus.

ANOSIM showed a significant difference ($P < 0.05$) from the control samples for three treatments – the dried samples and the two short-term acid treatments (Table 2). R_{ANOSIM} values are moderately high for the dried and 4M acid treated samples (0.64 and 0.68 respectively) and highest for the 1M acid treated samples (0.84), indicating a marked difference from the control. Of the experiments identified by ANOSIM, only one shows a significant difference in TI-DWT (dried samples, $P < 0.05$) and one shows a significant difference in Shannon-Wiener H (1M acid, $P < 0.05$).

The community differences between the control and the three experimental treatments identified by ANOSIM do not show a consistent pattern (Fig. 2). Of the most abundant taxa, *Heleopera petricola* is much reduced in the 4M acid treatment but only slightly reduced in the dried treatment and slightly elevated in the 1M acid treatment. *Hyalosphenia elegans* is markedly reduced in the dried samples but shows little change in the acid treated samples. The most consistent feature is a distinct loss of *Euglypha rotunda*, but this is a rare taxon even in the control sample.

Table 1. Experimental treatments applied.

Sample No.	Treatment	Replicate
1	Control	1
2	Control	2
3	Control	3
4	Control	4
5	Dried	1
6	Dried	2
7	Dried	3
8	Dried	4
9	Nutrient enrichment (0.8 g/sample)	1
10	Nutrient enrichment (0.8 g/sample)	2
11	Nutrient enrichment (0.8 g/sample)	3
12	Nutrient enrichment (0.8 g/sample)	4
13	Nutrient enrichment (0.4 g/sample)	1
14	Nutrient enrichment (0.4 g/sample)	2
15	Nutrient enrichment (0.4 g/sample)	3
16	Nutrient enrichment (0.4 g/sample)	4
17	Acid, long-term (H_2SO_4 pH 2)	1
18	Acid, long-term (H_2SO_4 pH 2)	2
19	Acid, long-term (H_2SO_4 pH 2)	3
20	Acid, long-term (H_2SO_4 pH 2)	4
21	Acid, short-term (1M H_2SO_4)	1
22	Acid, short-term (1M H_2SO_4)	2
23	Acid, short-term (1M H_2SO_4)	3
24	Acid, short-term (1M H_2SO_4)	4
25	Acid, short-term (4M H_2SO_4)	1
26	Acid, short-term (4M H_2SO_4)	2
27	Acid, short-term (4M H_2SO_4)	3
28	Acid, short-term (4M H_2SO_4)	4

Table 2. Results of ANOSIM of testate amoebae data and level of significance (ns = not significant).

Treatment	R_{ANOSIM}
Dried	0.65 ($P < 0.05$)
Fertilisation (higher dose)	0.32 ns
Fertilisation (lower dose)	0.49 ns
Acid treatment	0.48 ns
Short term- 1M acid	0.84 ($P < 0.05$)
Short term- 4M acid	0.68 ($P < 0.05$)

DISCUSSION

There is little obvious consistency of response between the experiments. The changes identified do not allow easy generalisation about the relative robustness of different amoebae taxa. The ANOSIM method used here shows there is a significant difference between the control and the dried sample and the control and the short-term acid treated samples. This does not *necessarily* mean that the difference is due to the experimental treatment. An alternative hypothesis is that there was some prior difference in the samples. Some taxa are apparently more abundant in the treated samples including some which are rare or absent in the control such as *Assulina seminulum* and *Nebela tincta* in the dried samples or *Centropyxis ecornis* in the 4M acid treated samples. An absolute increase in the abundance of these taxa could not be explained by a selective preservation affect; however this shows a relative change in their abundance which might be explained by a loss of other, more sensitive tests. Pre-treatment differences in the samples do appear unlikely given the very thorough attempt to homogenise the *Sphagnum* beforehand, the random attribution of samples to experimental treatment and the lack of a significant difference with the other three treatments. The most likely explanation therefore remains the experimental treatments.

One factor contributing to the subtle nature of the response may be the composition of the amoebae community in this site with a relatively small proportion of those taxa whose tests are likely to be most susceptible to decomposition. The most abundant taxa (*Hyalosphenia papilio*, *Archerella flavum*) have solid, secretion tests which previous work has suggested are resistant to decomposition. Tests constructed of idiosomes account for less than 16% of total tests in the control, and the largest proportion of these are *Assulina* taxa (7%) whose tests are reinforced with an organic cement and are generally considered very resistant to decomposition. *Euglypha* taxa only comprise 3% of the tests in the control samples. Another factor may be the timescale of these experiments. Although long by the standards of laboratory taphonomy experiments, the experimental period is very short compared to the palaeoecological record.

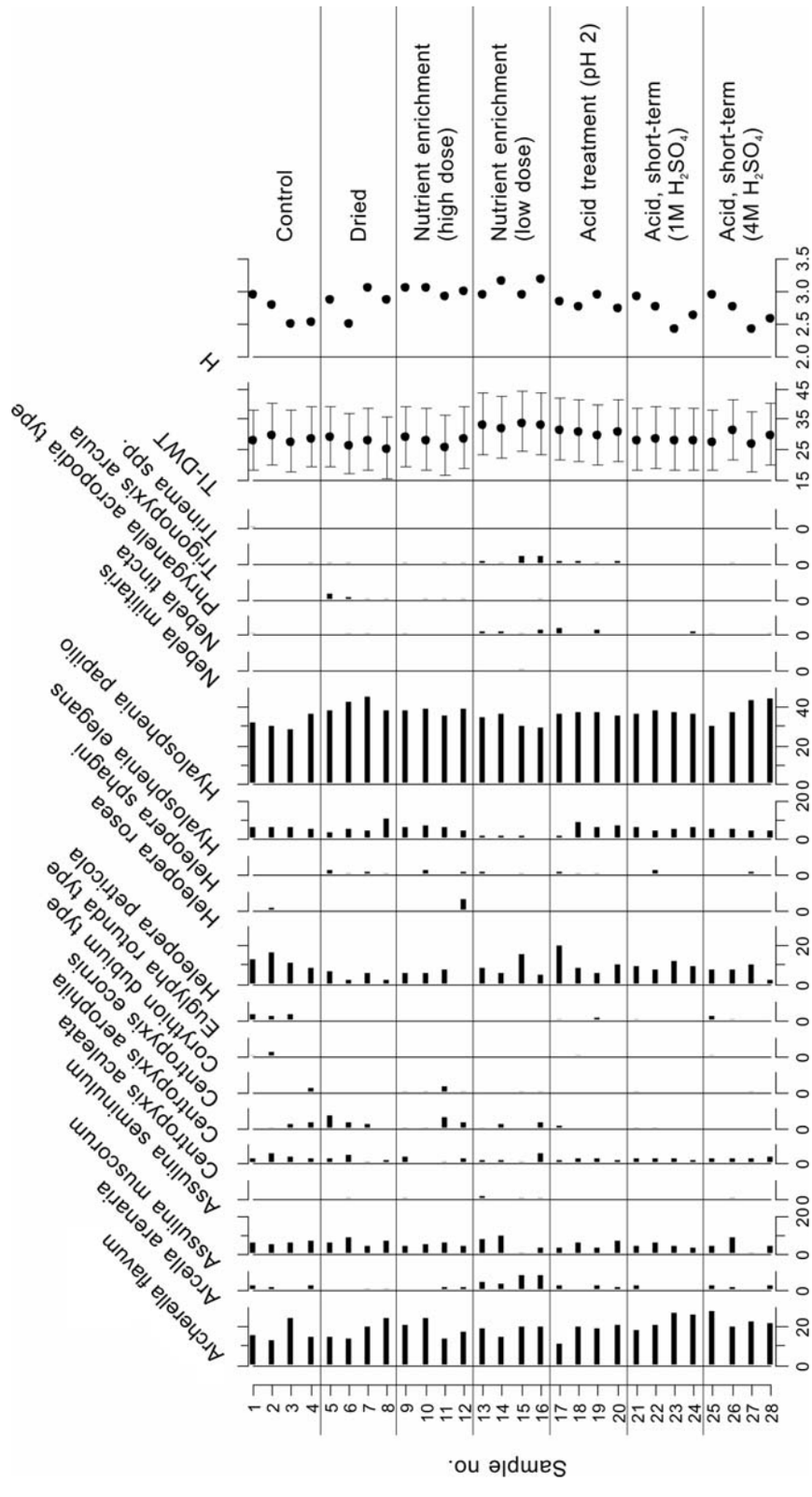

Fig. 1. Testate amoebae data showing major taxa, inferred water table (TI-DWT) with boot-strapped error estimates and Shannon-Weiner 'H' diversity index.

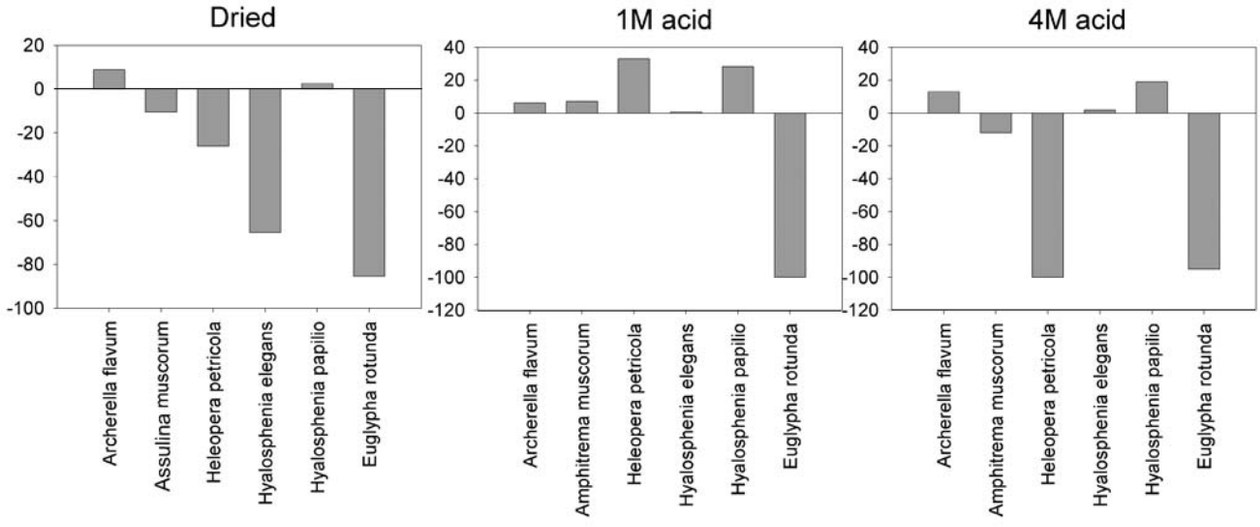

Fig. 2. Percentage change in abundance of selected testate amoebae taxa, showing taxa > 5% of total in control sample plus *Euglypha rotunda*.

Implications of the results

These results show the apparent sensitivity of tests to treatment with strong mineral acids and to desiccation. Swindles and Roe (2007) have shown that strong acids can lead to severe impacts on tests over very short periods of time. The acids used here are weaker and the response less clear-cut. The experimental scenario is not representative of the real-world situation of tests exposed to weak organic acids over a longer period. The long-term, lower-concentration, acid-treatment experiment did not show a significant response, although it is possible that this is simply a matter of limited time period. The result here supports previous work in suggesting the possibility of excess acidity affecting test preservation, but as in previous work the nature of the evidence is limited.

Arguably the more interesting result is the apparent sensitivity of tests to desiccation. This supports previous modern and palaeoecological observations (Charman *et al*. 2001, Roe *et al.* 2002). The most likely explanation for this impact is that dry conditions encourage microbial attack on tests leading to enhanced decompostion. This implies that many susceptible tests may be lost during dry periods in a peatland's history, and that this may preferentially affect certain taxa. In these experimental samples this has lead to a significant increase in inferred water table depth. This presents a cause for concern when using testate amoebae to reconstruct palaeohydrology, particularly in dry periods, suggesting that differential test preservation may lead to inaccurate paleohydrological reconstruction. The direction of change in TI-DWT suggests that dry periods may be exaggerated. Considering these results and those of previous studies it seems that the most likely indicator of preservation problems may be a loss of *Euglypha* taxa. Future palaeoecological studies will need to carefully evaluate the potential impact of selective test decomposition on their results, particularly during dry periods. These results may also have relevancy to the curation of samples for testate amoebae analysis, suggesting that drying of samples may be inappropriate.

Future experimental work

The experiments in this study suggest the sensitivity of tests to acidity and desiccation. However these initial experiments only go a limited way towards resolving the controls on test preservation and much further work will be needed. From the results here, some suggestions can be made for future experimental studies.

One of the factors in the rather subtle response to the experimental treatments here is almost certainly the composition of the amoebae community studied. For future work it may be valuable to select samples which contain a high proportion of those taxa thought most susceptible to decomposition (such as *Euglypha* taxa).

The samples used in this experiment were taken from subsurface peat, which was probably deposited around 100–150 years ago (Payne 2005). This approach was used to ensure that no living amoebae remained in the samples. It is possible that many of the most sensitive tests had already decomposed before the start of the experiment reducing the apparent response. Future experiments could address this by using surface samples treated (perhaps by radiation) to kill the live amoebae but not damage tests. In these experiments changes in the amoebae communities were assessed using percentage data. A more sensitive approach might be to use absolute abundances, however as most palaeoecological studies use percentage data it is this result which is the more relevant to the palaeoecological record.

A possible complicating factor in this study is heterogeneity in the samples. Future studies should choose samples and adopt homogenisation techniques to minimise this risk and possibly increase the replication of the experiments to minimise the impact of any heterogeneity. It may also be valuable to store samples at a more realistic temperature than used in this study.

Future experiments will need to investigate a full range of possible environmental variables and use scenarios which are as realistic as possible and experiments which are as long-term as possible. As only a sub-sample of the material in these experiments was analysed in this study it may prove worthwhile to re-examine these samples at some point in the future.

Acknowledgements. This study was initiated while the author was supported by a QMUL Westfield Studentship and completed during the duration a University of Manchester Humanities Research Fellowship. Thanks to Jeff Blackford, Tom Finnie and Pete Ryan for useful discussion and to two anonymous referees for comments on a previous draft.

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Received on 24th July, 2007; revised version on 24th September, 2007; accepted on 26th September, 2007