Rearing parasitoids: what parameters are the most important?

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Highlights

- Temperature and diet are mentioned in 90% of papers where parasitoids are reared.
- No relationship was found between the mentions of sex-ratio and those of other parameters.

Abstract

Parasitoid population dynamics are sensitive and colonies can become extinct unexpectedly and for reasons unclear. A systematic word count in abstracts as well as full texts allowed us to determine which environmental parameters were seen as important by researchers in experiments where parasitoids are reared. The experimental parameters investigated were sex-ratio, temperature, humidity, pressure, diet, environment structure, host quality and population dynamics. Temperature and diet were by far the most often mentioned parameters. Sex-ratio yielded less than a quarter as many hits as temperature, despite its importance for colony survival. Physical properties like pressure and humidity were seldom mentioned. No relationship was found between the number of mentions of sex-ratio, or the presence of sex-ratio (at least 1 mention), and the mentions or the presence other parameters. A more complete search and analysis method could yield more decisive results. Parameters like temperature, diet, environment structure and sex-ratio appeared together more frequently. Researchers working with parasitoids should take note of these parameters and perform sex-ratio counts whenever practical. As evidenced by the complexity of parasitoid population ecology, further studies would be needed for each parasitoid.

Keywords

parasitoid, rearing, parameters, factors, experiment, laboratory, text mining

Introduction

Biological control has been a topic of scientific investigation for over a century. Yet, given the number of different techniques (predation, parasitism) and the sensitivity in application (different climates, trophic interactions), research continues at pace. The *Centre for Agriculture and Bioscience International* (CABI) is an intergovernmental organization which has been working on biological control measures, and more specifically invasive species control for many decades. A recent subject of investigation is the Fall Armyworm (*Spodoptera frugiperda*, a devastating pest of crops, see Rwomushana et al. 2018, pp.23-27) and its control using natural enemies, in this case parasitoid wasps. Parasitoids reproduce using a host to support the development of their eggs and larvae. The host remains alive during this time. The larvae may develop inside the host or on its surface. When development is through, the host is killed. Most parasitoid species are of the suborder Apocrita (Hymenoptera). Part of the quarantined facilities at CABI Switzerland were dedicated to studying the parasitism rate of *S. frugiperda* by two wasps: the braconid *Chelonus insularis* and the ichneumonid *Eiphosoma laphygmae* (Allen et al. 2020). However, during the course of the experiments, the parasitoid populations fluctuated greatly. In particular, sex-ratio was very unstable, even leading to the extinction of one of the *C. insularis* colonies.

A precursory review of the related literature revealed that sex-ratio is known to be unstable in parasitoids. Sex-ratio is expected to follow a 1:1 average since that would give the best evolutionary fitness, yet for parasitoids this is not the case. Godfray (1994) describes several experiments where sex-ratio was skewed without explanation. Since it is the female's decision whether to lay a male or female egg, factors that influence behaviour can be suspected as well. For instance, one paper showed that rapid changes in barometric pressure discouraged flight in female of two *Trichogramma* species (Fournier et al. 2005), which has consequences on parasitoid density in a patch. A review of sex-ratio theory and possible explaining factors is found in Wajnberg et al. (2007). The authors also propose models for optimal behaviour. Quicke (1997) warns that primary sex-ratio is difficult to determine in parasitoids because there might be differential mortality between male and female eggs or larvae. Finally, a review on sex-ratio in ichneumonoids was done by Smart & Mayhew (2008). Sex-determining mechanisms, differential mortality, superparasitism and sperm limitation are put forward as potential explanatory parameters. The authors conclude that among all the rules having an influence on sex-ratio, each species must use a specific mix. Indeed, in the case of CABI, different items were investigated, including the climate regulation appliances, but no cause could clearly be identified for the collapse of the *C. insularis* colony. In any case, some of these parameters could be controlled relatively easily (temperature) while others would have required more advanced equipment (pressure). Some other parameters would be beyond control entirely, given the way the experiment was set up.

Therefore, it was decided to proceed to a systematic review of papers that include parasitoid rearing as part of their experimental protocol, following the idea that experimental parameters which are the most frequently mentioned are generally perceived as the most essential to control for the success of experiments. With this information, researchers who are working with parasitoids could implement measures to better control population dynamics in their colonies.

In this review, we establish the frequencies of occurrence of certain **rearing-related parameters** in the text of selected Open Access articles. We then compare the frequencies depending on the occurrence of terms related to sex-ratio.

Methods

The new *Web of Science* website was selected[1] to search for papers. The search string targeted papers in which a parasitoid was reared, either to study it, study its interactions with another species, or for the sake of finding the best rearing methods. This narrowing down was done by searching the titles and abstracts for specific terms: `rear` and all its forms (`reared`, `mass-rearing`), `parasitoid`, words that might denote experimental parameters (`factor`, `effect`). The *OR* clause on Web of Science is not exclusive. Variations on parasitoid such as `parasite`, `hyperparasitoid` or `superparasitism` did not improve the quality of matches and were discarded. The same was done for words targeting specific topics of research such as `diet` or `factitious hosts`. The query was iteratively refined, manually assessing the relevance and specificity of results until the best compromise was achieved.

Less relevant results were excluded by enabling 'exact search'. This functionality prevents so-called 'fuzzy matching' in which related matches are injected into the query according to unknown and uncontrollable rules.

The final search was performed on February 10, 2021 with the query (see Annex 1):

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( TI=(reared) OR AB=(rearing) OR AB=("to rear") ) AND AB=(parasitoid*) AND ( AB=(mass) OR AB=(population*) OR AB=(method*) OR AB=(factor*) OR AB=(effect*) OR ( AB=(environment*) AND AB=(parameter*) ) )
```

This search yielded 913 highly relevant hits. The records were imported into Citavi and the full texts were downloaded automatically wherever possible. By the nature of this process, only papers available in Open Access were included. Of the 495 with the full text available online, 201 were downloaded. For the remaining 712 papers, the abstracts were collected into a secondary corpus (see Annex 2).

In these corpuses, the presence of an experimental parameter of interest is evaluated through the number of occurrences of related words. In text-analysis terms, the parameters are called **topics** and the words are looked for with search expressions. These expressions might use wildcards (*). The algorithm returns all matching tokens (units of text). Which parameters are to be investigated can be based on Schneider (2009). Temperature is a determining parameter for the development of insects. Humidity, pressure and other climate-related parameters are also important in that they must match the studied species' habitat. Other parameters, not based on the measure of ambient physical properties, constitute further parameters that researchers may have taken note of. Sex-ratio bias can lead to extinction if too high, suggesting that experimenters might often track this measure, if only for practical reasons. According to Wajnberg et al., the female parasitoid's host search strategy is informed by cues about certain environmental factors. While the experiments set up at CABI are not really concerned with host search (the parasitoids are confined in cages), the spatial characteristics of the environment such as its patchiness (a measure of heterogeneity) have an influence on parasitoid behaviour. A host's quality - mostly determined by its size - has also been shown to influence egg-laying decision, as well as search strategy. Although *C. insularis* is an egg parasitoid, and thus host size is not a concern, some

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[1] https://clarivate.com/webofsciencegroup/solutions/web-of-science/
Web of Science is the successor to *Web of Knowledge* and uses the same database.

papers may include it for their insect[2]. The respective diets of the parasitoid (usually honey) and the host (often standardized) should also be included. Finally, there are intrinsic factors as well. The branch of biology concerned with population dynamics has produced general rules (such as local-mate-competition or LMC) that must apply to parasitoid rearing.

The list of topics and expressions to match is established as follows:

- Sex-ratio: `sex_ratio, *male_biased`
- Temperature: `degre*, °*, temper*, warm*, heat*`
- Humidity: `humid*, damp, hygro*`
- Pressure: `Pa, bar, atmos*`
- Diet: `diet*, nutri*, food, forag*`
- Structure: `patch*, habit*, distan*, locat*, wind`
- Hosts: `host_quality, host_size`
- Dynamics: `LMC, competition, *ism_rate*, life_histor*, foundress*`

Certain characters, words or phrases have to be transformed. In particular, phrases that must be interpreted as one token (eg. "sex ratio" should not be counted as "sex" and "ratio"). Very short words and punctuation are also removed. See Annex 3 for the exact text sanitization steps.

The corpuses are analysed in R 4.0.2 with RStudio 1.3 and the text-analysis package Quanteda 2.1.2. The graphs are created in Python 3.9 with Pandas 1.2.1 and Seaborn 0.11.1 (see References).

The count data is subjected to separate statistical tests for each corpus. In abstracts, where words are fewer but chosen more carefully, the distribution of counts is broken down according to the presence or not of sex-ratio related terms and ANOVA is used to test whether that same variable influences the distribution. In papers, chi-square is used to test whether the presence of sex-ratio influences the presence of other topics. The number of occurrences of each topic after the number of occurrences of sex-ratio is modelled through linear regression. Finally, the association of topics is evaluated with a ratio of co-occurrence.

Results

The corpuses were assembled from papers where parasitoids were reared. This is a sufficiently narrow scope, despite the overlap between parasitoid rearing and insect rearing in general. The total counts are shown in Table 1 and plotted in Figure 2, while the per-paper counts can be found in Annex 4. The two corpuses show similar distributions, with exceptions. Physical properties like pressure and humidity, as well as host quality, were the least often mentioned, totalling together around 5% of hits in each corpus. Environment structure, population dynamics and sex-ratio make up a second group, amounting to about 25% of hits in both corpuses. Diet shows a much higher prevalence in full texts, where scores closer to temperature with 30% than in abstracts where it is closer to sex-ratio with 18%. The remaining topic, temperature, accounts for 53% of hits in abstracts and 39% in full texts.

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[2] Egg parasitoids do have ways other than size of determining a host's quality.

One could argue that the evolution of the sex-ratio in a colony is the most important parameter for its survival, yet it does not achieve first place. The overbearing presence of temperature-related words shows that researchers give it a lot of importance. It is indeed essential for the development of insects, governing their development time and lifespan, but this is not specific to parasitoids. To extract actionable information, we can look for relationships between each topic and sex-ratio.

In abstracts, the distribution of topics according to the presence of sex-ratio is shown in Figure 3. Sex-ratio is present in 23% of abstracts. An ANOVA yields a p-value of 0.297, meaning that the null hypothesis that the presence of sex-ratio has no influence on the distribution of parameters cannot be invalidated.

In papers, chi-square is used to test the relationship between the presence of a topic and the presence of sex-ratio. The test yields a p-value of 0.339, again indicating that a relationship is extremely unlikely. A correlation plot (Figure 4) shows no strong relationship between topic either, with an absolute maximum of 0.266 occurring between host quality and sex-ratio. Finally, a linear regression of each topic against sex-ratio yields large residuals and p-values, eliminating all credibility of a relationship between the two.

All that is left is to check which parameters occur more frequently together. In papers, the frequency of appearance of topics in pairs can be compared to their total frequency of appearance. This is the Jaccard index, the ratio of the intersection over the union. The result is shown in Figure 5. The most strongly associated topics are temperature, diet, environment structure and population dynamics. Sex-ratio is also strongly associated with diet and temperature.

Tables

Table 1: Total parameter mentions

Parameter	Name	Keywords	Abstract	Full texts
Sex-ratio	sexratio	`sex_ratio, *male_biased`	319	693
Temperature	temperature	`degre*, °*, temper*, warm*, heat*`	1463	2748
Humidity	humidity	`humid*, damp, hygro*`	53	208
Pressure	pressure	`Pa, bar, atmos*`	10	43
Diet	diet	`diet*, nutri*, food, forag*`	536	2118
Environment structure	structure	`patch*, habit*, distan*, locat*, wind`	167	510
Host quality	hosts	`host_quality, host_size`	65	146
Population dynamics	dynamics	`LMC, competition, *ism_rate*, life_histor*, foundress*`	181	577

Figures

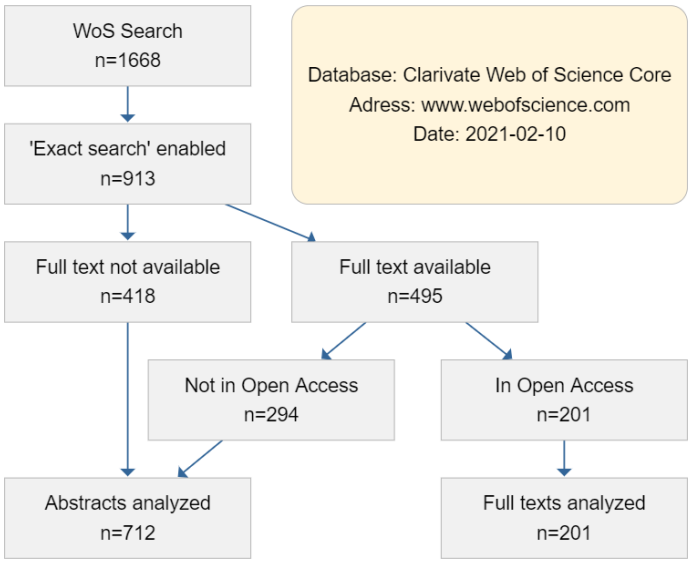

Figure 1: PRISMA diagram of the paper selection process

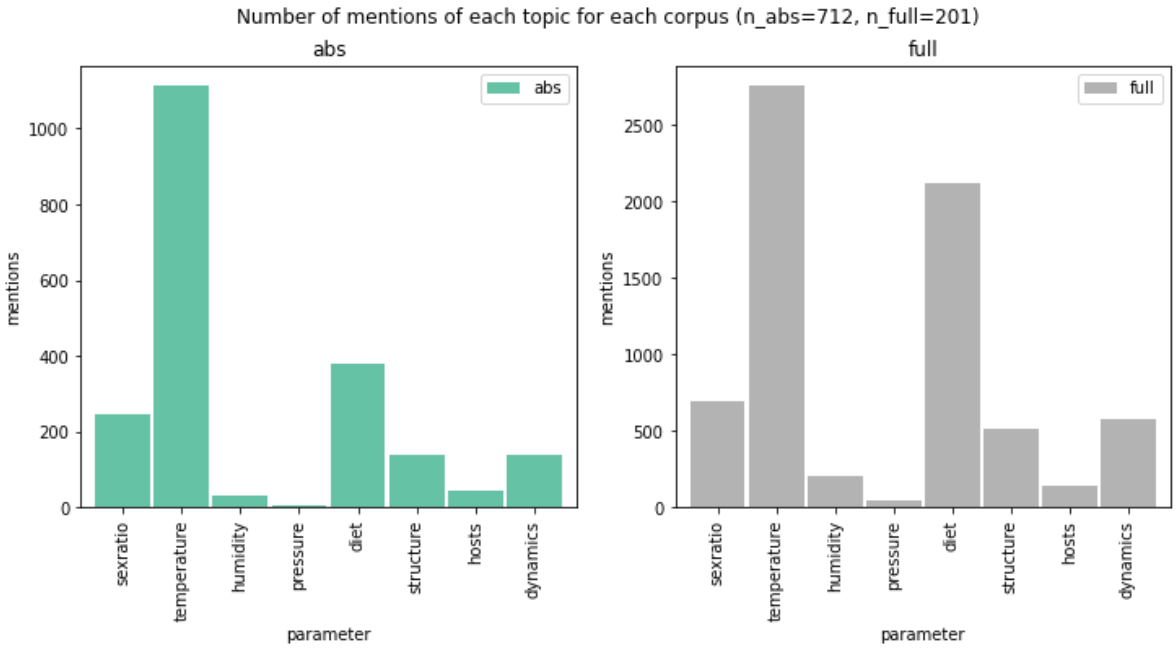

Figure 2: Number of mentions of each topic in abstracts (abs) and full texts (full).

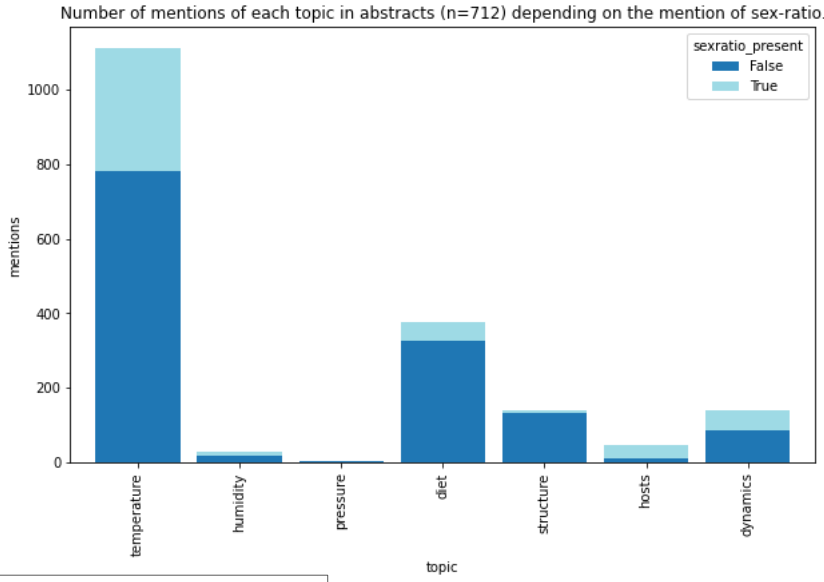

Figure 3: Mentions of each topic in abstracts (n=712) according to the mention of sex-ratio.

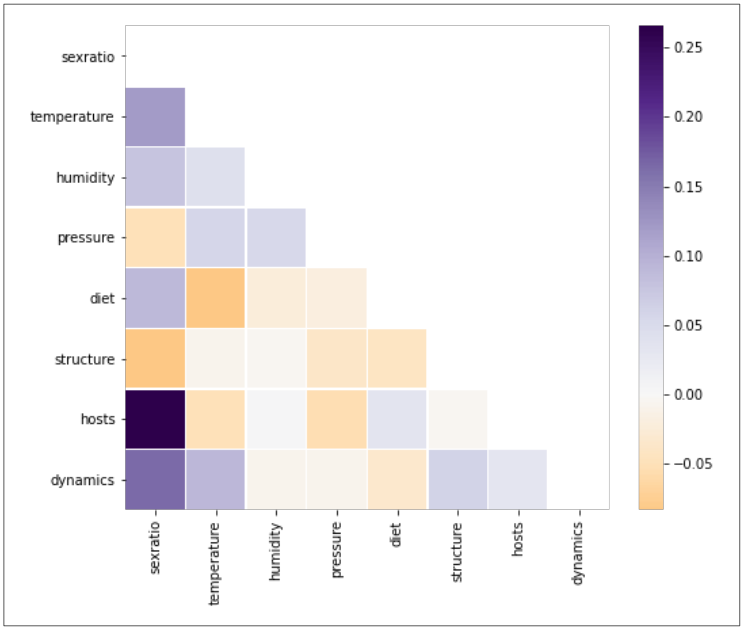

Figure 4: Pearson correlation between parameters in full texts (n=201).

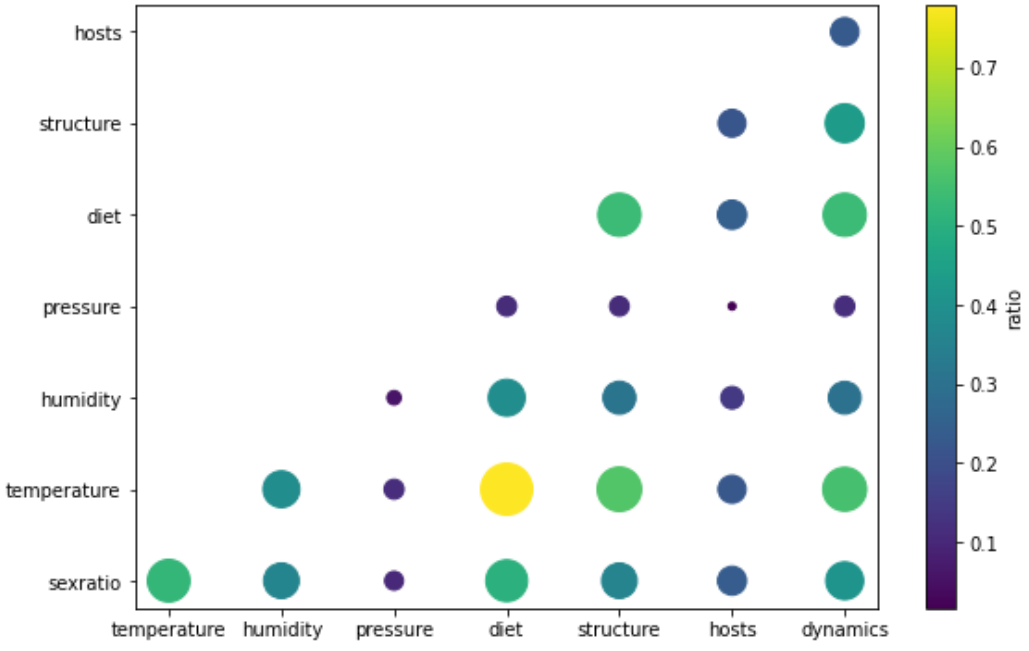

Figure 5: Jaccard index for each topic pair in papers (n=201).

Conclusions

No meaningful relationship was found between the number of mentions of sex-ratio and that of other topics. We can still draw a few conclusions with respect to the initial question: in rearing parasitoids, what parameters are seen as the most important? Thanks to the per-topic count, we have determined that some are more prevalent than others in articles concerned with parasitoid rearing. Parasitoid-specific topics such as host quality did not achieve particularly high scores. This suggests that the same parameters that are important in general insect rearing are important for parasitoid rearing as well. Aside from their primary role in parasitoid development, parameters may be included for other reasons. In the context of a reproducibility crisis, a very general parameter like temperature can be put forward as a disclosure of sorts, as an attempt to be as transparent as possible.

The lack of a decisive trend in our data can in part be attributed to our methodology. As many as 294 papers were excluded from this analysis because they were not in Open Access. This is one of the items that could easily be improved upon. Topic occurrence has shown its limits. First, the list of keywords, as carefully crafted as it might be, is a limiting factor. Ideally it should be balanced so as not to give a topic more chances simply because it is associated with more general keywords. The probability of each of those words to appear in any given text is intrinsic to the language used, in our case scientific English. In a narrow field like parasitoid rearing, word frequencies do not show what underlying ideas motivated their use[3]. Second, whether a topic is mentioned in relation to rearing success or something else cannot be determined. A topic might be mentioned but not investigated at all. Another way of counting would be required to go past this problem, such as natural language processing which extracts meaning from words' positions inside sentences and their relationships.

In reality, as shown by the preliminary round-up of the literature on parasitoids, environmental and population-level parameters can only partially explain the evolution of a population. They vary greatly in nature and, as a product of evolution, the host-parasitoid system should be able to cope with these fluctuations. Successful rearing of parasitoids is a function of several factors and as outlined by Smart & Mayhew (2009), each species might require its own dedicated study to see which ones are the most important. Researchers concerned with rearing their insects in good conditions should look at temperature and diet, and perform sex-ratio counts whenever practical. The role of environment structure must be studied more closely, so that the best way to split a colony between cages can be determined. For instance, cages could be connected with one another. This is surely a limiting factor in any experiment, since it depends on the space and materials available. Wajnberg et al. (2007) explain how environmental cues can direct parasitoid behaviour. In chapter 16 (pp359-371), they show how individual strategies might be elaborated using simple 'wait functions' approximating the ideal Bayesian behaviour. This suggests that the cues received by the insects could be used beneficially. Lastly, genetic sex-determining mechanisms can vary among species. The best insurance against colony extinction is to maintain multiple inbred lines (p276) and inject new genetic material regularly, from the field or another colony (Schneider, 2009).

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[3] Contrary to what we might find in a corpus of political tracts, for instance. In text analysis, this measurement is called 'keyness'.

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Annex

1. Query

```
  ( TI=(reared) OR AB=(rearing) OR AB=("to rear") )
AND AB=(parasitoid*)
AND (      AB=(mass)
      OR   AB=(population*)
      OR   AB=(method*)
      OR   AB=(factor*)
      OR   AB=(effect*)
      OR ( AB=(environment*) AND AB=(parameter*) )
    )
```

Database: Web of Science
Address: www.webofscience.com
Date: 2021-02-10
Hits: 1668

Or use the following link ('exact search' must be enabled manually):

https://www.webofscience.com/wos/woscc/general-summary?q=W3sidCI6IiggVEk9KHJlYXJlZCkgT1IgQUI9KHJlYXJpbmcpIE9SIEFCPShcInRvIHJlYXJcIikgKVxuICBBTkQgQUI9KHBhcmFzaXRvaWQqKVxuICBBTkQgKCAgICAgQUI9KG1hc3MpXG4gICAgICBPUiAgIEFCPShwb3B1bGF0aW9uKikgXG4gICAgICBPUiAgIEFCPShtZXRob2QqKVxuICAgICAgT1IgICBBQj0oZmFjdG9yKikgXG4gICAgICBPUiAgIEFCPShlZmZlY3QqKVxuICAgICAgT1IgKCBBQj0oZW52aXJvbm1lbnQqKSBBTkQgQUI9KHBhcmFtZXRlcioqKSApXG4gICAgKSIsIm1vZGUiOiJhZHZhbmNlZCJ9

2. Papers included

Set 1: Papers available in full: pastebin.com/raw/WH5JHvbh

Set 2: Papers with only the abstract available: pastebin.com/raw/qxhy44su

3. Text treatment

Full articles were cleaned-up according to these steps (in order):

1. Remove species abbreviations
2. Replace hyphens with underscores
3. Compound temperatures
4. Remove numeric values
5. Remove parentheses and their contents
6. Remove symbols and apostrophes
7. Remove all text after the word 'References'
8. Remove lone characters
9. Remove very short words
10. Remove 'al'
11. Compound expressions

4. Resources

Code for the analysis in R: pastebin.com/raw/9PJFddF8

Topics count per paper for abstracts: pastebin.com/raw/naD6YUJh

Topics count per paper for full texts: pastebin.com/raw/WSuw502z

The co-occurrence count: pastebin.com/raw/anxVZ3Wp

5. Screenshots

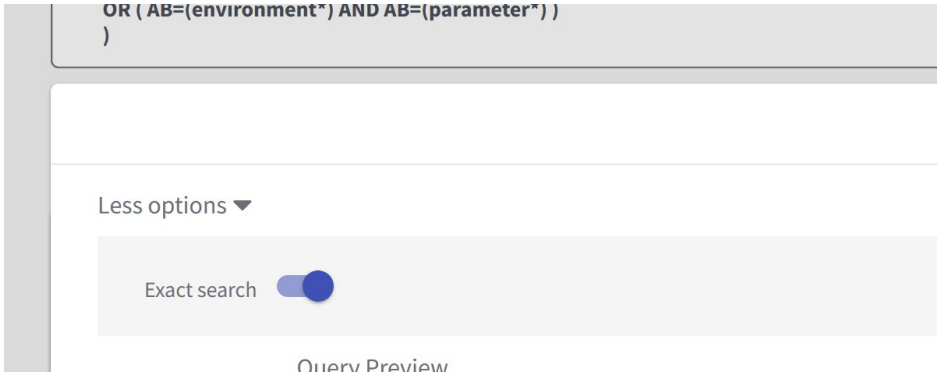

Fig. A5: When performing a search on Web of Science, 'exact search' must be enabled to exclude records that only loosely match.