

# AntMap Ver. 1.2

Constructing genetic linkage maps by ant colony  
optimization algorithm

Developed by  
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**AntMap ver. 1.2**

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***Source code and executables of AntMap are available under GNU general public license (GPL) at <http://cse.naro.affrc.go.jp/iwatah/antmap/index.html>. AntMap comes with ABSOLUTELY NO WARRANTY, and you are welcome to redistribute it under certain conditions; see Appendix A (GNU General Public License) for details.***

**AntMap Website:**

<http://cse.naro.affrc.go.jp/iwatah/antmap>

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## **Brief Overview**

Locus ordering is an essential procedure in genome mapping. When the number of loci is large, it is quite difficult to determine the optimum order with an exhaustive search of all possible orders. The problem of searching for the optimum order has been recognized as a special case of the traveling salesman problem (TSP), i.e., given a set of cities and distances for each pair of them, find a roundtrip of minimal total length visiting each city exactly once. In recent years, Ant Colony Optimization (ACO) (Dorigo and Stützle 2004), which is a set of algorithms inspired by the behavior of real ant colonies, has been successfully used to solve discrete optimization problems, such as TSP. We developed a novel system based on ACO for locus ordering in genome mapping (Iwata and Ninomiya *in preparation*). In our system, loci and absolute value of log likelihood (or recombination fraction) between loci were regarded as TSP cities and distance between cities, respectively. We tested the system using a simulated segregation population, and found it is highly efficient for linkage grouping as well as locus ordering in genome mapping (Iwata and Ninomiya 2004).

To commoditize our newly-developed system, we developed software named AntMap for constructing linkage map by the system. AntMap performs segregation test, linkage grouping and locus ordering, and constructs a linkage map quite rapidly and nearly automatically. Rapidity of the algorithm based on ACO enables us to conduct a bootstrap test of estimated order. With the aid of this software, researchers can save their time and labor, and can obtain a linkage map whose reliability is indicated by bootstrap values. Another advantage of AntMap is the fact that AntMap is open source; that is, source code and executables of AntMap are available under GNU General Public License (GPL) (see Appendix A for details). Java and C++ objects that code our newly-developed system will be utilized effectively for other applications as well as AntMap.

### **Ant Colony Optimization (ACO)**

See Iwata and Ninomiya (in prep.).

### **New Algorithm for Locus Ordering**

See Iwata and Ninomiya (in prep.).

## System Requirements

- Windows, Linux, Solaris or Mac OS.
- Java 2 Platform Standard Edition (J2SE) Java Runtime Environment (JRE) (ver. 1.4 or higher).

If you have no J2SE JRE (ver. 1.4 or higher) already installed on your system, you should install the JRE to execute AntMap on your system. To install JRE, please see <http://java.sun.com/javase/downloads/index.html>.

## Installation

1. Download the zipped file named “AntMap.zip”, which contains all the AntMap files. The file is available at <http://cse.naro.affrc.go.jp/iwatah/antmap>.

The files contained in “AntMap.zip” are as follows:

AntMap.jar     executable jar file

(It can be executed on any OS on which the JRE is installed)

AntMap.exe     native executable file for Windows

AntMap-linux   native executable file for Linux

AntMap-solaris native executable file for Solaris

AntMap-macx   native executable file for Mac OS X

sample.raw     a sample file

AntMapSource.jar     archive file which contains source codes

AntMapDoc.jar     archive file which contains Javadocs

license.txt     text file which describes GNU GPL

Manual.pdf     user’s guide for AntMap.

2. Extract the folder named “AntMap” from the “AntMap.zip”.
3. You can put the extracted folder anywhere you want.
4. (Optional) If your OS is Windows, make a short cut to “AntMap.exe” on your desktop for convenience.

## Input File Format

Input file format of AntMap is identical to \*.raw files required by MapMaker (Lander et al. 1987). A concise description of the format of \*.raw files can be found in <http://www.rfcgr.mrc.ac.uk/Registered/Help/mapmaker/>.

AntMap can analyze data derived from progeny of several types of crosses, including:

- (1) F2 intercross
- (2) F2 backcross (e.g., BC1)
- (3) Recombinant inbred lines by self-mating
- (4) Doubled haploid lines

The first line of input file indicates the crossing types as:

```
data type xxxx
```

where xxxx should be one of the followings:

```
f2 intercross
```

```
f2 backcross
```

```
ri self
```

```
dh
```

The current version of AntMap does not support two types of cross, F3 intercross by self-mating (`f3 self`) and recombination inbred lines by sib-mating (`ri sib`), which are supported by MapMaker EXP.

## Brief Tutorial

### Step 0: Start AntMap



Fig. 1

When you use AntMap on Windows, start AntMap with double-clicking the “AntMap” icon (Fig. 1). For other operating systems (i.e., platforms), See below.

#### Box 1.

##### Linux and Solaris

Before executing “AntMap-linux” or “AntMap-solaris”, you should change mode of these files to be executable. To do that, type

```
chmod 755 AntMap-xxxx
```

on your command line system (“xxxx” should be “linux” or “solaris”). After changing the mode of files, you can execute AntMap by clicking the “AntMap-linux” or “AntMap-solaris” file icon.

##### Mac OS X

Note that you can execute “AntMap-macx” from the command line, but cannot execute by clicking the “AntMap-macx” file icon.

AntMap can also be executed by using the executable jar file “AntMap.jar” on any platforms (Linux, Solaris and Mac OS as well as Windows). To execute the jar file, run:

```
java -jar AntMap.jar
```

on your command line system. Some platforms may have bindings already set up such that you can execute the jar file just by clicking on the “AntMap.jar” file icon, which will run the command line equivalent. Note that you should change mode of the jar file to be executable when you are on “Linux” or “Solaris” platforms as described in Box 1.

**Step 1: Open an input file.**

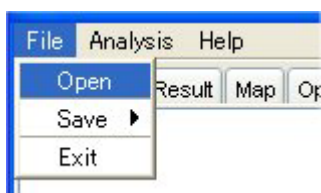


Fig. 2

Open an input file in MapMaker format (\*.raw) through “File-Open” menu (Fig. 2). Here, open “sample.raw” contained in the “antmap” folder.

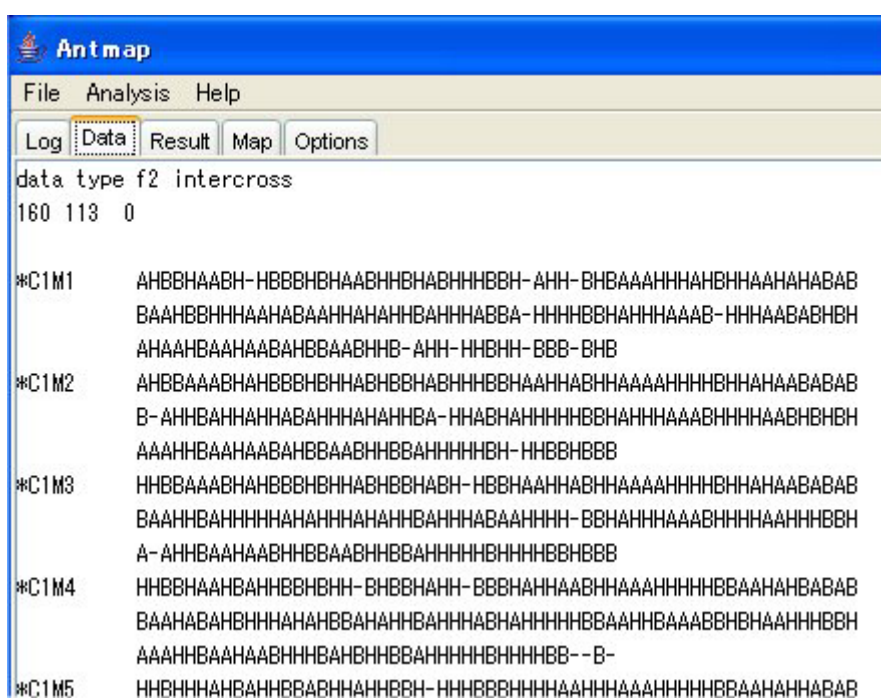


Fig. 3

After opening the file, contents of the file will appear in the “Data” panel (Fig. 3).

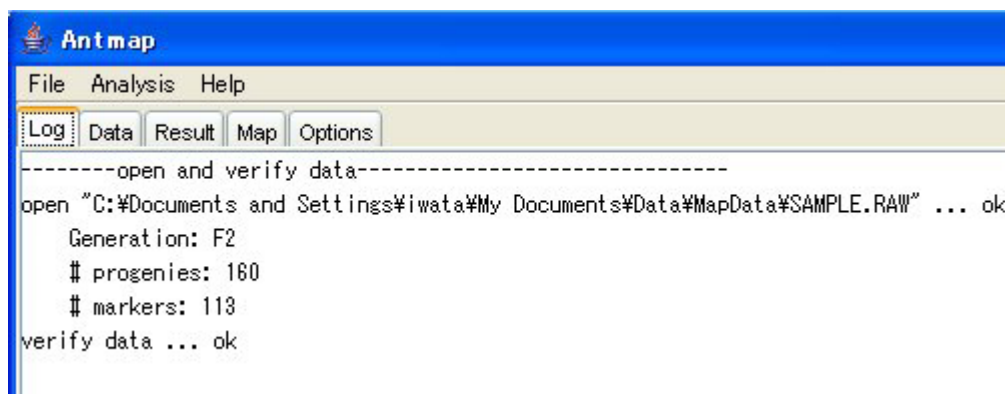


Fig. 4

Click the “Log” tab, and you can see a summary of the input data (Fig. 4).



**Step 2: Segregation ratio test.**

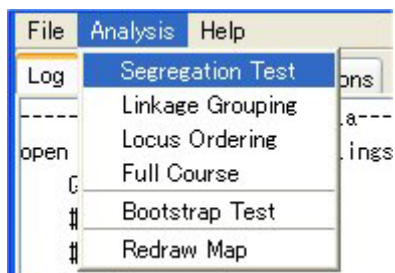
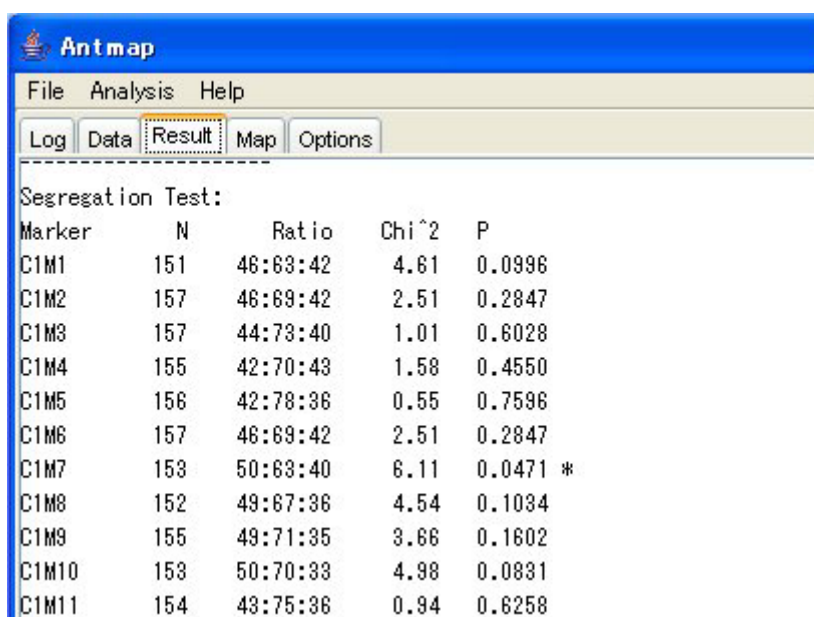


Fig. 5

Select "Segregation Test" from the "Analysis" menu (Fig. 5). Then you can see the results of segregation ratio tests in the "Result" panel (Fig. 6).



Antmap

File Analysis Help

Log Data Result Map Options

Segregation Test:

Marker	N	Ratio	Chi <sup>2</sup>	P
C1M1	151	46:63:42	4.61	0.0996
C1M2	157	46:69:42	2.51	0.2847
C1M3	157	44:73:40	1.01	0.6028
C1M4	155	42:70:43	1.58	0.4550
C1M5	156	42:78:36	0.55	0.7596
C1M6	157	46:69:42	2.51	0.2847
C1M7	153	50:63:40	6.11	0.0471 *
C1M8	152	49:67:36	4.54	0.1034
C1M9	155	49:71:35	3.66	0.1602
C1M10	153	50:70:33	4.98	0.0831
C1M11	154	43:75:36	0.94	0.6258

Fig. 6

### Step 3: Linkage grouping

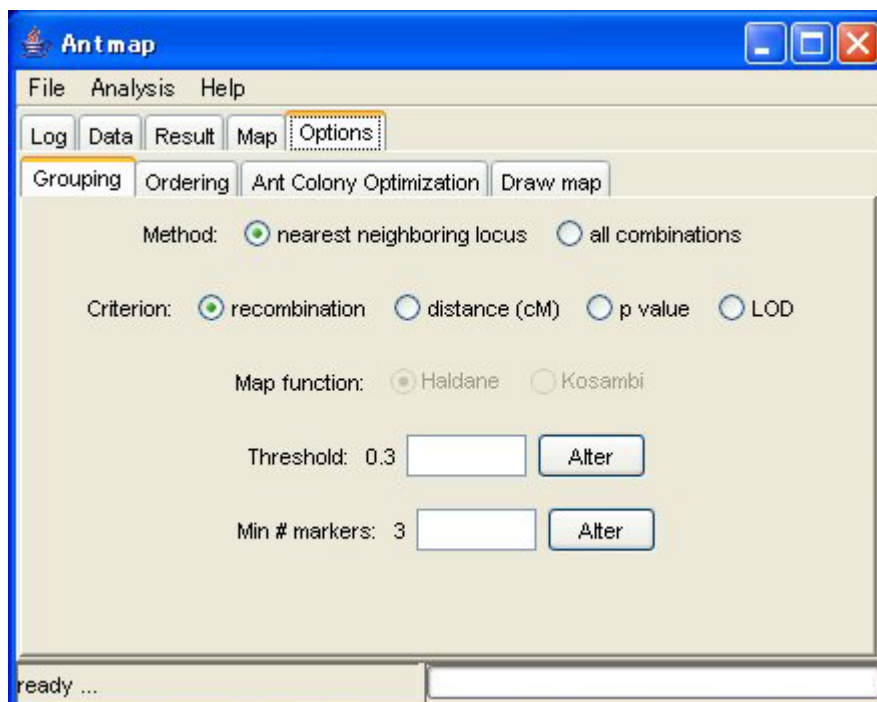


Fig. 7

Click the “Options” tab. Then you can see the “Grouping” option panel (Fig. 7).

You can choose one of the two grouping methods: “nearest neighboring locus” and “all combinations”. The former makes a group by sequentially combining a locus which shows the smallest recombination value against it. This algorithm has been implemented by MAPL (Ukai et al. 1991). The latter will produce similar results with “group” command of MapMaker.

You can also choose the grouping criterion, threshold value and the minimum number of markers for a single group.

Here, we will keep these options unchanged except for the threshold value.

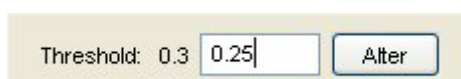


Fig. 8

Type “0.25” into an input area, and push the “Alter” button. Then you can change the threshold value from 0.3 to 0.25.

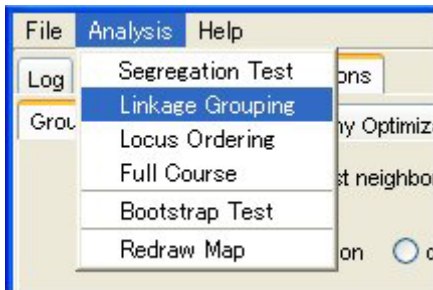


Fig. 9

Select the “Linkage Grouping” from the “Analysis” Menu. Then you can see the results of linkage grouping in the “Result” panel (Fig. 10).

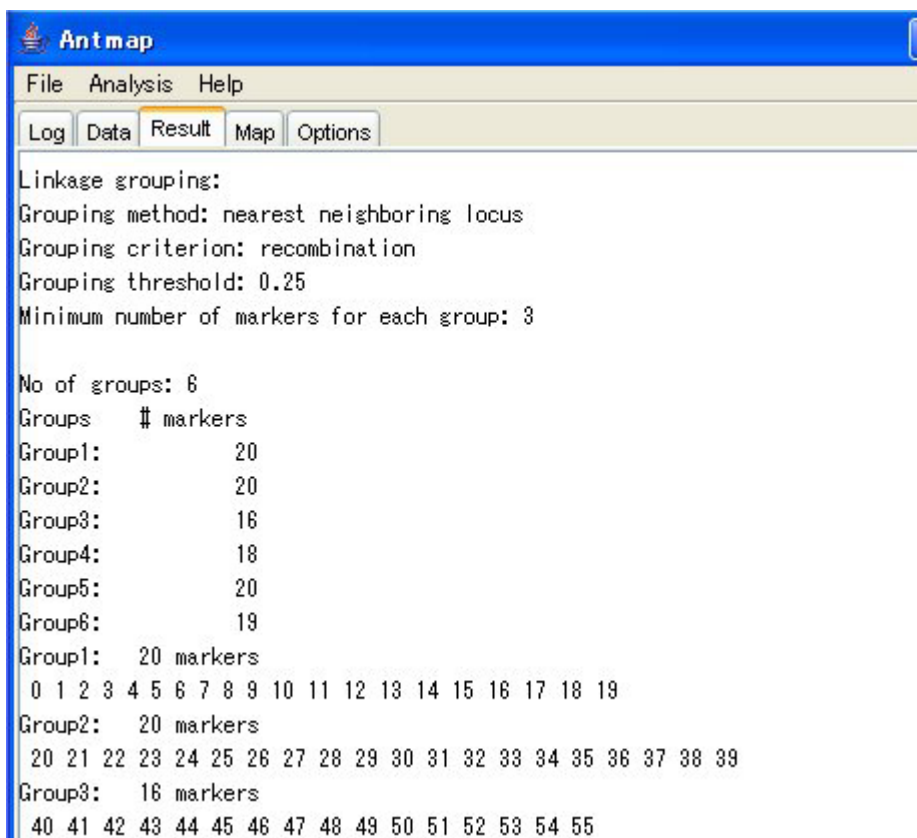


Fig. 10

When you analyze your data, you may not be able to achieve a good separation of markers to linkage groups from the start. In such a case, please find a good set of the threshold value, criterion and method through try-and-errors.

## Step 4: Locus ordering

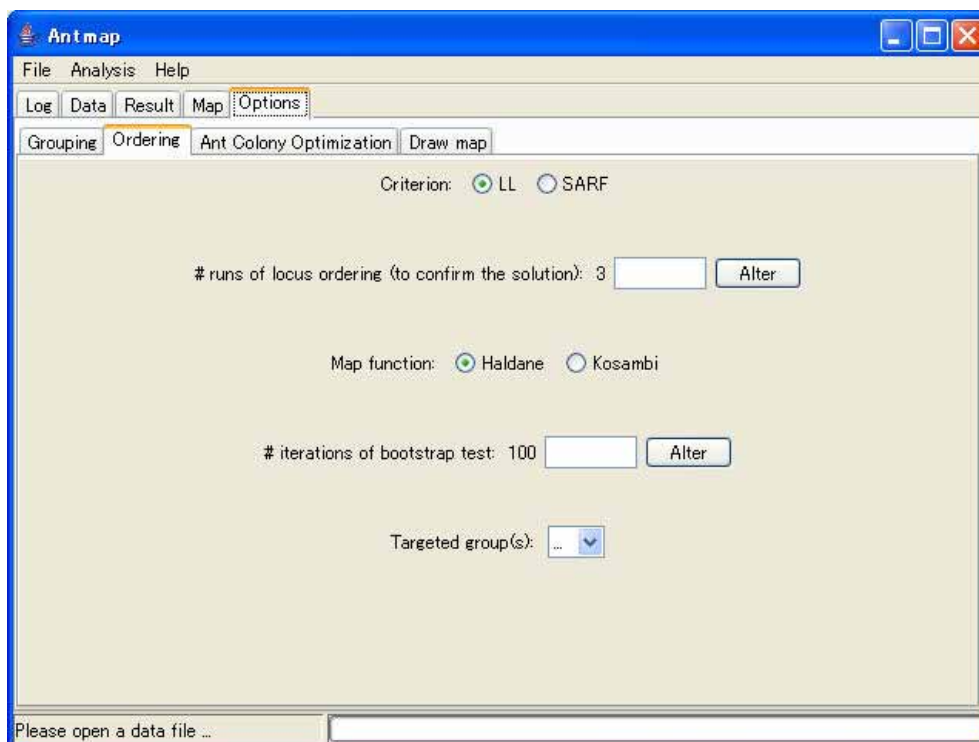


Fig. 11

Click the “Options” tab, and click the “Ordering” tab. Then you can see the “Ordering” option panel (Fig. 11).

In the locus ordering, you can choose one of the two criteria: “LL” and “SARF”. “LL” is an abbreviation for “Log Likelihood”. “SARF” is an abbreviation for “Sum of Adjacent Recombination Fractions” (Liu 1998). AntMap will search a locus order which maximizes log-likelihood or minimizes “SARF”.

You can also choose the number of runs of locus ordering. You can find the meaning of this option in the “AntMap Options” section of the AntMap use’s manual.

A map function for calculating a map distance between adjacent markers can be selected from “Haldane” (Haldane 1919) or “Kosambi” (Kosambi 1944) functions.

Here, we will keep these options unchanged.

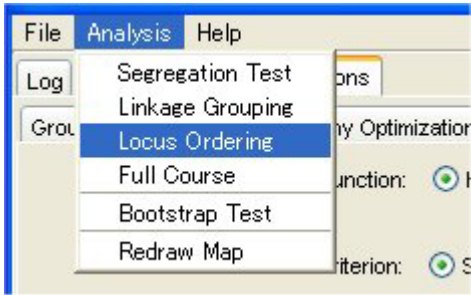


Fig. 12

Select the “Locus Ordering” from the “Analysis” Menu. Then you can see the results of locus ordering in the “Result” panel (Fig. 13).

Index	Locus	Position
0	C1M1	0.00
1	C1M2	7.07
2	C1M3	9.77
3	C1M4	18.24
4	C1M5	22.47
5	C1M6	29.32
6	C1M7	40.11
7	C1M8	43.36
8	C1M9	53.10
9	C1M10	55.55

Fig. 13

You can also obtain a graphic of linkage map in the “Map” panel (Fig. 14).

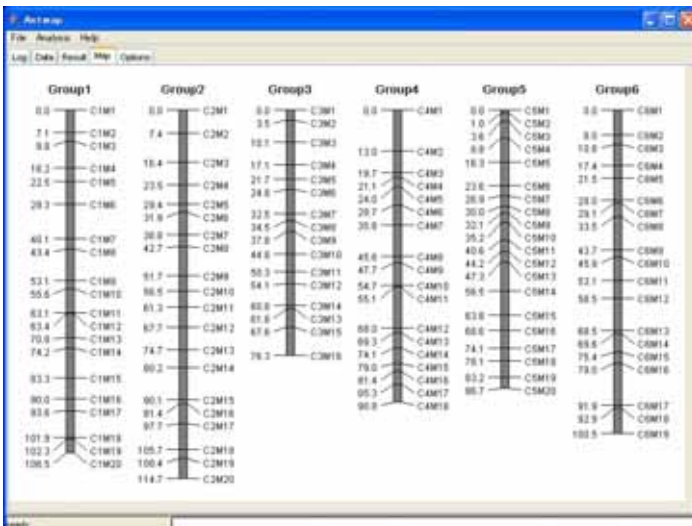


Fig. 14

### Step 5: One-step mapping

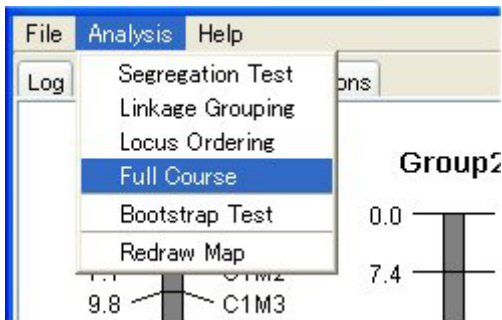


Fig. 15

Select “Full Course” from the “Analysis” Menu. Then, you can overall process from segregation ratio test (Step 2) to locus ordering (Step 4) at once.

### Step 6: Redraw a linkage map

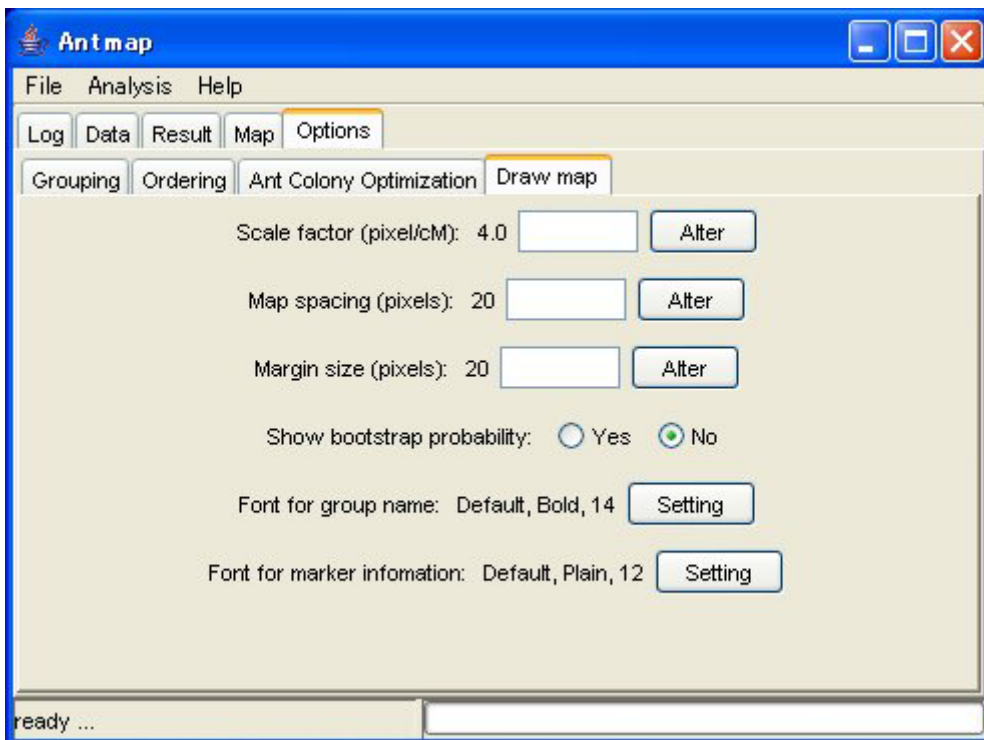


Fig. 16

Click the “Options” tab, and click the “Draw map” tab. Then you can see the “Draw map” option panel (Fig. 16).

Here, we will change the “Scale factor” option. Drawing size of linkage map can be changed through this option. Here, type “2” into an input area, and click the “Alter” button (Fig. 17).

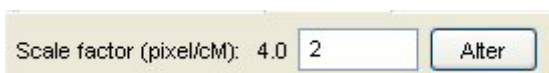


Fig. 17

After changing the option value from 4 to 2, select “Redraw Map” from the “Analysis” menu. Then, you can obtain a smaller linkage map than one obtained previously (Fig. 18).

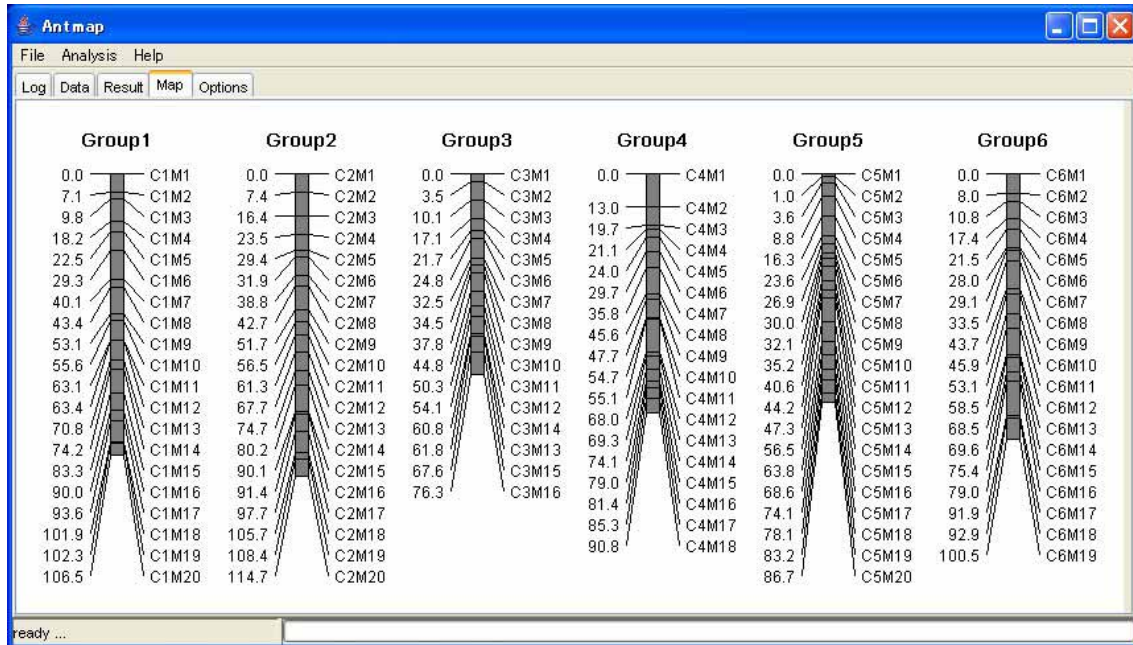


Fig. 18

### **Step 7: Bootstrap test for locus order**

You can evaluate the reliability of estimated locus order by using bootstrap test. Bootstrap test (or bootstrapping) is a method for estimating the sampling distribution of an estimator by resampling with replacement from the original sample. In a bootstrap test, a random sample of size  $n$  is drawn from the original sample of size  $n$ , and estimates are obtained from the random sample. After repeating (iterating) this operation many times (e.g., 100-1000 times), the stability of estimates (e.g., standard error or confidence interval of estimators) is evaluated. For the details of bootstrap test, please see a good textbook such as Manly (1998). In the bootstrap test for locus order, we can obtain probability that a locus is located at its estimated order (Liu 1998).

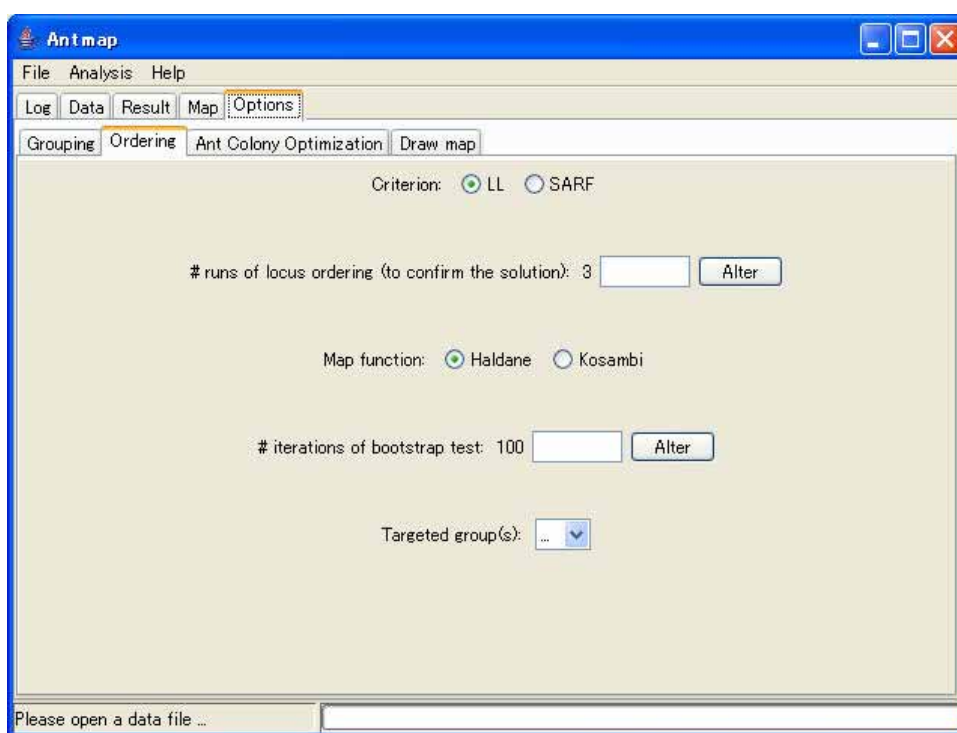


Fig. 19

Click the “Options” tab, and click the “Ordering” tab. Then you can see the “Ordering” option panel (Fig. 19).

You can change the number of iterations (repeats) of bootstrapping. To get a good estimate of percentage of correct locus order, 100 may be sufficient.

You can also choose a group which is targeted in the bootstrap test. Here, we will choose only Group3 to save our time (Fig. 20).



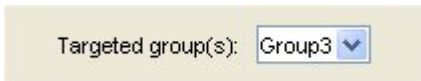


Fig. 20

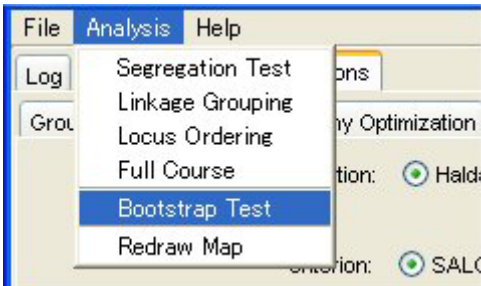


Fig. 21

Select the “Bootstrap Test” from the “Analysis” Menu (Fig. 21). Then you can see the results of bootstrap test for locus order in the “Result” panel (Fig. 22).

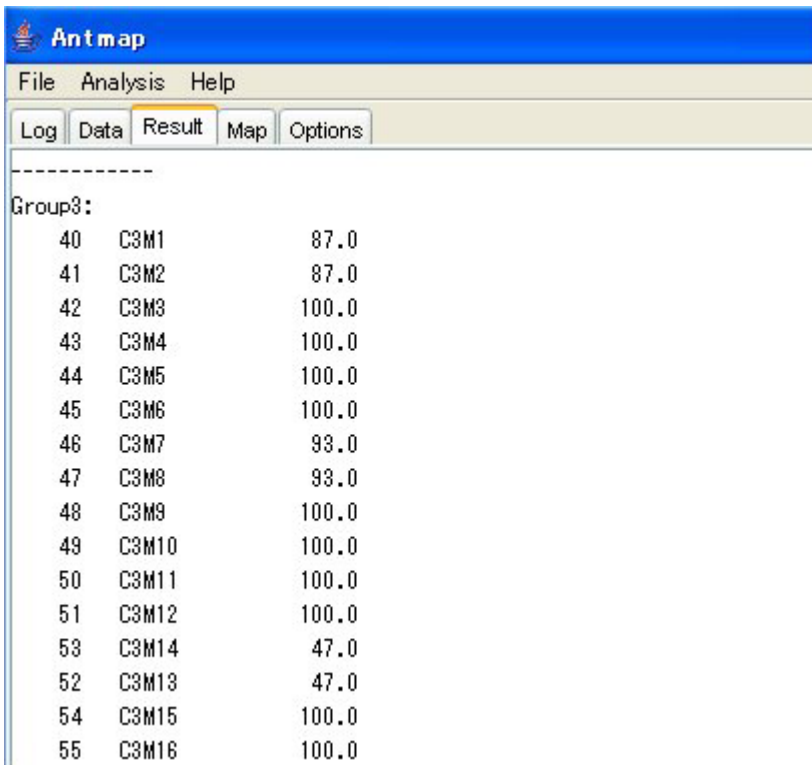
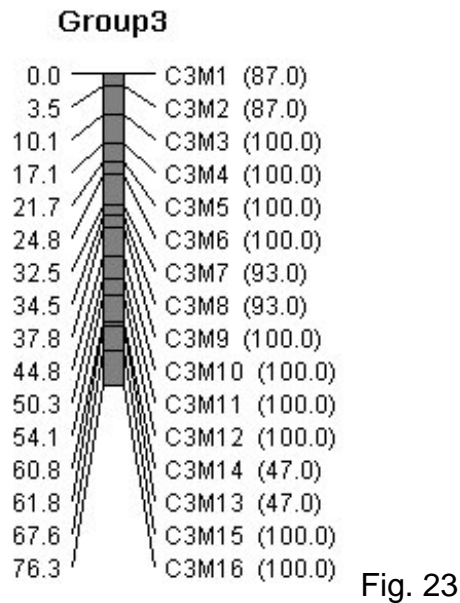


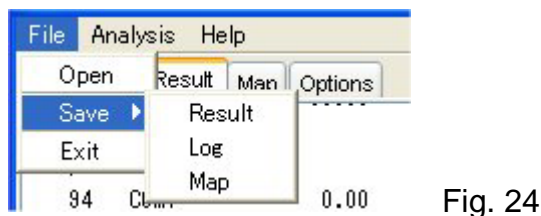
Fig. 22

You can also obtain a graphic of linkage map with bootstrap values in the “Map” panel (Fig. 23).



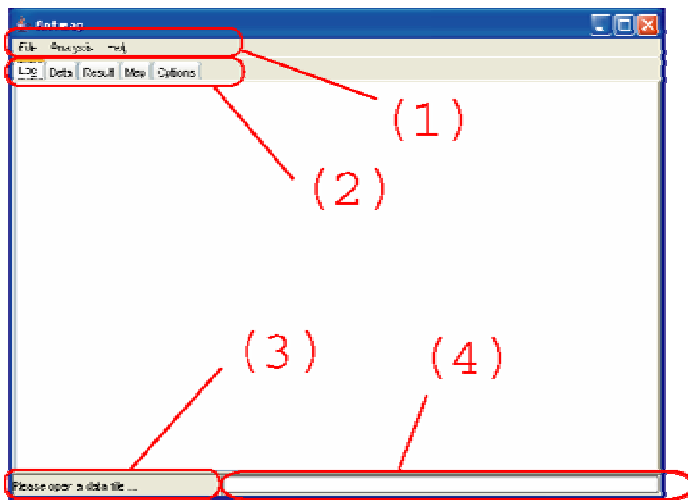
**Caution! : The bootstrap test for all linkage groups may take long time even by high-end PC. Thus, you have better set your computer to perform this test at your lunch time or after going home.**

**Step 8: Save results of linkage mapping**



You can save information in “Result”, “Log” and “Map” panels through the “Save” submenu in the “File” Menu. The information in “Result” and “Log” is saved as a text file. The information in “Map” (i.e., a graphic of linkage map) is saved as a JPEG (\*.jpg) file.

## AntMap Window



### (1) AntMap Menus

See the next section.

### (2) Tabs for Panels

#### 1. "Log" Panel

Analysis log is output in this panel.

#### 2. "Data" Panel

Input data are shown in this panel.

#### 3. "Result" Panel

Results of linkage mapping are shown in this panel.

#### 4. "Map" Panel

A graphical image of estimated linkage map is shown in this panel.

#### 5. "Options" Panel

Setting of options for linkage mapping can be checked and changed through this panel.

### (3) Status Indicator

Status of the software is indicated here as a text message.

### (4) Progress Bar

A bar which indicates progress in calculation requested by the user.

## AntMap Menus

AntMap operates interactively through menu selections made by a user. Explanations for the menus are as follows.

### File menu

- | **Open**  
    Open an input file
- | **Save**
  - | Result  
    Save contents in the "Result" panel.
  - | Log  
    Save contents in the "Log" panel.
  - | Map  
    Save a graphic in the "Map" panel.
- | **Exit**  
    Quit AntMap

### Analysis menu

- | **Segregation Test**  
    Conduct segregation ratio test
- | **Linkage Grouping**  
    Conduct linkage grouping
- | **Locus Ordering**  
    Conduct locus ordering
- | **Full Course**  
    Conduct overall process of linkage mapping  
    (segregation test, linkage grouping, locus ordering)
- | **Bootstrap Test**  
    Conduct bootstrap test for locus order
- | **Redraw Map**  
    Redraw a graphics of linkage map

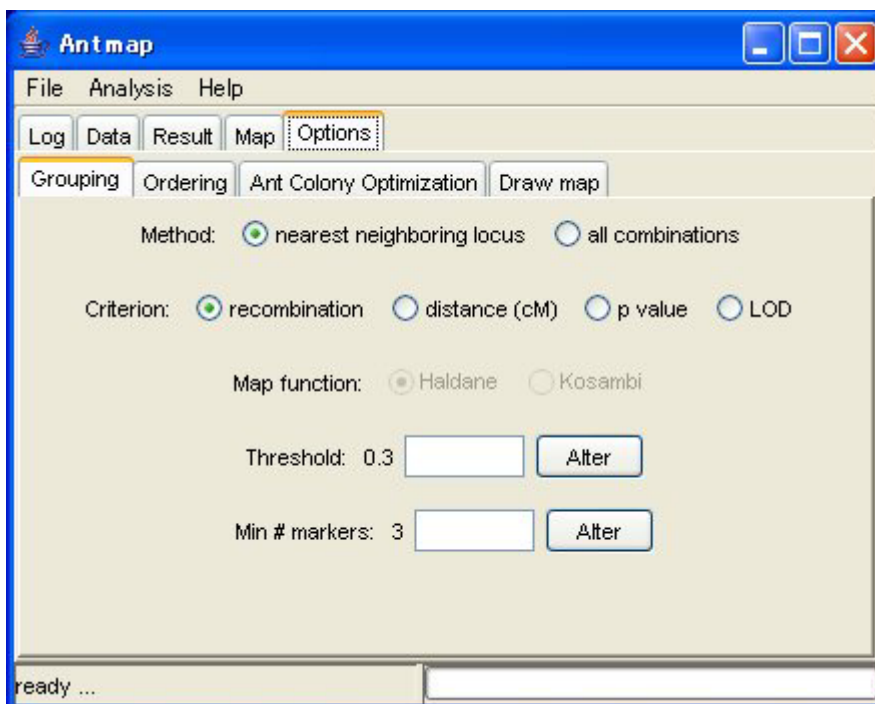
### Help menu

- | **About**  
    Show the version information of AntMap.
- | **About GPL**  
    Show GNU General Public License (GPL)

## AntMap Options

A user can check setting of options in linkage mapping and change them through the “Options” panel. In this panel, there are four child-panels which can be switched by clicking tabs. Explanations for the options are as follows.

### “Grouping” options



#### *Method – Method for linkage grouping*

You can choose one of the two grouping methods: “nearest neighboring locus” and “all combinations”. The former makes a group by sequentially combining a locus which shows the smallest recombination value against it. This algorithm has been implemented by MAPL (Ukai et al. 1991). The latter will produce similar results with “group” command of MapMaker.

#### *Criterion – Criterion for linkage grouping*

You can choose one of the four grouping criteria: “recombination”, “distance (cM)”, “p value” and “LOD”. “recombination” and “distance (cM)” are recombination value and map distance (in centi-Morgan) between two loci, respectively. “p value” is a p value for chi-square test statistic under the assumption of no linkage between two loci and no segregation distortion for both loci. “LOD” is a LOD score which is defined as the following formula:

$$\text{LOD score} = \log \left[ \frac{\text{probability of the observed data with a given recombination value}}{\text{probability of the observed data under no linkage}} \right].$$

*Map function – Map function to be used*

When you choose “distance (cM)” as the criterion, you can choose one of two mapping functions for calculating map distance. “Haldane” is the function derived by Haldane (1919) and “Kosambi” derived by Kosambi (1944).

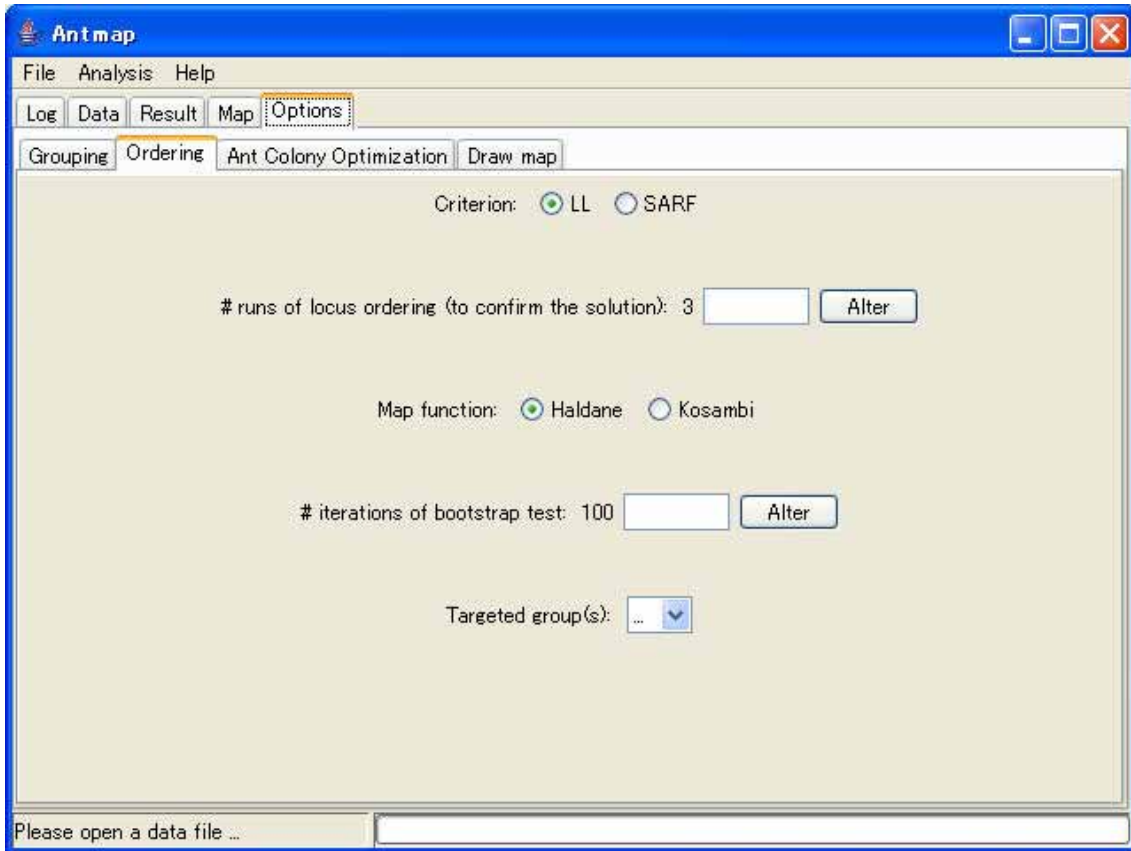
*Threshold – Threshold value for grouping*

When you choose “LOD” as the criterion, this value is used as the lower threshold for grouping. When you choose the other criteria, it is used as the upper threshold. **Please set this value appropriately for the criterion chosen by you.** For example, the default value “0.3” may be appropriate for the “recombination” criterion, but is too severe for the “distance (cM)” criterion and is too loose for the “p value” and “LOD” criteria.

*Min # markers – Minimum number of markers in a linkage group*

A linkage group which contain less than this number of markers is not considered as a linkage group. When you set this number to 1, an independent locus (i.e., a locus which is not linked to the other loci) is considered as a single linkage group.

## “Ordering” options



### *Criterion – Criterion for locus ordering*

You can choose one of two optimization criteria for locus ordering. “LL” is an abbreviation of “Log Likelihood”, and “SARF” is an abbreviation of “Sum of Adjacent Recombination Fractions” (Liu 1998). AntMap will search a locus order which maximizes “LL” (i.e., minimizes the absolute value of “LL”) or minimizes “SARF”.

### *# runs of locus ordering – The number of runs of locus ordering to confirm the solution*

A meta-heuristics algorithm, such as ACO, does not necessarily reach the best solution of a given problem at all the times. Consequently, AntMap obtains a number of solutions by plural runs of searching (i.e., locus ordering) process, and then adopt the best one (i.e., the optimum order) as the conclusive solution (i.e., the estimated order). “# runs of locus ordering” is the number of runs of locus ordering to find the conclusive solution. When this number gets large, the chance to find the best solution gets large. But, the iterations may be very time consuming. In most cases (e.g., when the number

of markers in a single linkage group is less than 30), 3 may be sufficient.

*Map function – Map function to be used*

You can choose one of two mapping functions for calculating map distance between loci. “Haldane” is the function derived by Haldane (1919) and “Kosambi” derived by Kosambi (1944).

*# iterations of bootstrap test – Number of iterations in bootstrap test*

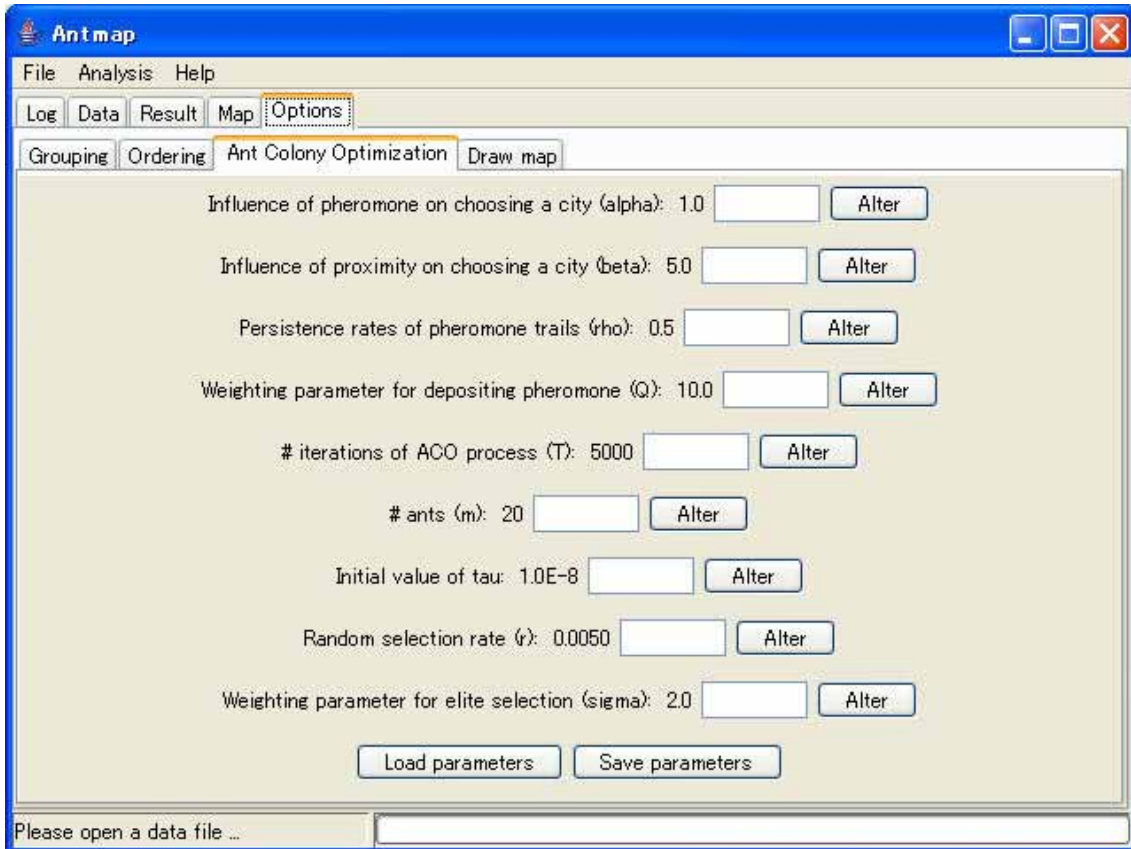
You can change the number of iterations (repeats) of bootstrapping. To get a good estimate of percentage of correct locus order, 100 may be sufficient.

*Targeted group(s) – Targeted group(s) in both locus ordering and bootstrap test*

You can choose a linkage group which is targeted in locus ordering and bootstrap test for locus order.



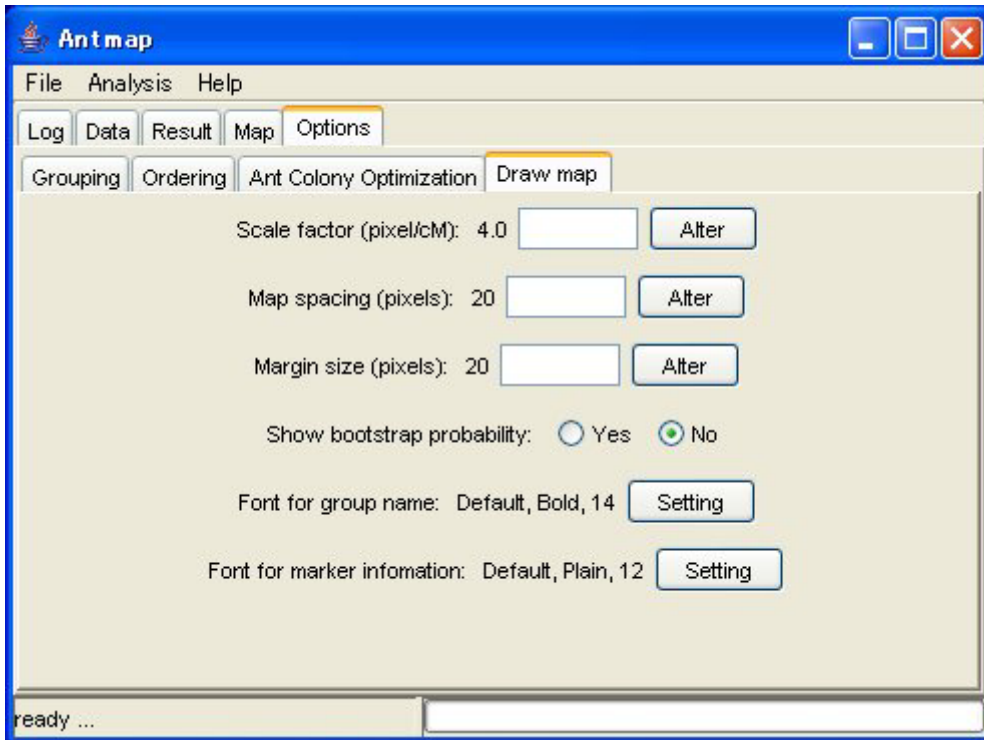
## “Ant Colony Optimization” options



These options are for parameters in Ant Colony Optimizations. Most users may not have to change these parameters, because they have been almost optimized for locus ordering problems (Iwata and Ninomiya, in prep). If you are interested in the effects of these parameters, please change them and perform locus ordering. You may be able to find out a better set of parameter values than the default setting. The parameter set adjusted by you can be saved to a file (click the “Save parameters” button) and loaded again (click the “Load parameters” button) in your subsequent analyses.

The details of these parameters are given in the “Ant Colony Optimization (ACO)” and “Locus Ordering by ACO algorithm” sections.

## ***“Draw Map” options***



### ***Scale factor (pixel/cm) – Scale for drawing a graphic of linkage map***

A larger value of this option makes distance between loci larger in the graphic, and vice versa. When you construct a high-density linkage map, it may be better to enlarge this value.

### ***Map spacing (pixels) – Space between linkage groups***

### ***Margin size (pixels) – Margin size in a graphic of linkage map***

You can adjust the size of margin and space in a graphic of linkage map.

### ***Show bootstrap probability – Show bootstrap probability of locus order***

When “Yes” is checked, bootstrap probabilities in bootstrap test of locus order are appeared in a graphic of linkage map.

### ***Font for group name – Font attributes for linkage group names***

### ***Font for marker information – Font attribute for marker information***

You can choose font attributes for linkage group names and marker information (i.e., marker location, name and bootstrap probability, through this option).

### **How to cite AntMap**

AntMap is open source software. However, please cite AntMap when you intend to publish results from AntMap. Now we are preparing a paper reporting our study related to AntMap. At the current moment, please cite AntMap as follows:

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