Appendix

Annex 1: life cycle of a typical Myxogastria

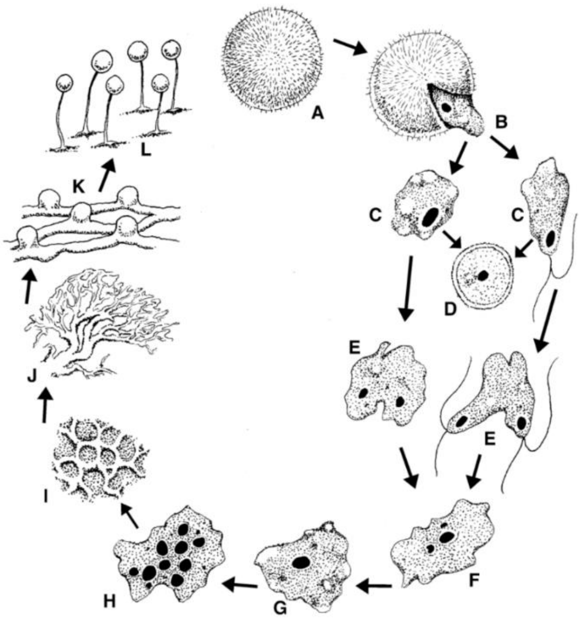

Figure 1: A – spore, B – germinating spore, C – mononuclear amoeboid phase, with (right) or without (left) whiptail, D – microcyst, E-F – fusion of two compatible amoebae to produce one cell, G – zygote, H – early plasmodium, I – sclerotium, J – part of a mature plasmodium, K – beginning of spore formation, L – mature fruiting body with spores still enclosed [11]

Annex 2: computer program

In the maze there are routes of 11, 15 and 23 square blocks long. The computer model assumes that for each route there is one pawn at the start. The pawns represent *P. polycephalum*. Each pawn tries to reach the end point via its own route. One pawn may move forward one block at a time. Therefore, the probability that a pawn in the model moves forward is always 1/3, since there are three pawns. The model determines again and again which pawn can take a step, until one pawn reaches the finish first and thus 'wins'. After this, the program puts the pawns back at the start and again determines which route wins. Because the program chooses 10 000 000 times which pawn takes a step and every time a pawn gets to the finish determines which route has 'won', it is made clear how often each route 'wins' in total.

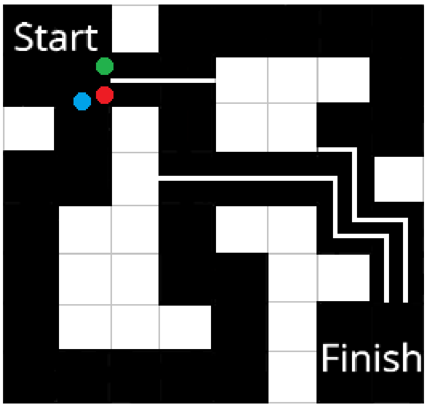

Figure 2: schematic representation of the route that pawns have to take in the model, the red pawn has to take 11 steps, the green 15 and the blue 23, the routes are separated by white lines

Annex 3: written program to calculate the probability of reaching via the shortest route

```
#include <iostream>
#include <time.h>

using namespace std;

int main()
{
   cout<<"Random Generator "<< "\n";

/* s is the short path, m is the medium path and l is the long path */
/* sTot is amount of taken steps on short path, mTot and lTot are the same for their paths */
/* tot is total amount of steps taken */
/* dS is the percentage of reaching the end point by the shortest path, dM and dL are the same for their paths*/

   int iRandom, s, m, l, sTot, mTot, lTot, tot;
   double dS, dM, dL;
   s=sTot=m=mTot=l=lTot=tot=0;
   dS=dM=dL=0.0;

/* initialize random number */
   srand (time(NULL));

   for (int i = 0; i < 10000000; i++) {
  /* generate random number between 1 and 3 */
   iRandom = rand() % 3 + 1;

   if (iRandom == 1) {s++;
      if (s==10){sTot++, s=0; tot++;}
   }
   if (iRandom == 2) {m++;
      if (m==14){mTot++, m=0; tot++;}
   }
   if (iRandom == 3) {l++;
      if (l==22){lTot++, l=0; tot++;}
   }
}

dS = 100.0*sTot/tot ;
dM = 100.0*mTot/tot ;
dL = 100.0*lTot/tot ;

cout<<"short "<< sTot<< ", "<<  dS <<"%" <<"\n";
cout<<"medium "<< mTot<< ", "<< dM <<"%" <<"\n";
cout<<"long "<< lTot<< ", "<< dL <<"%" <<"\n";

   return 0;
}
```

Annex 4: step-by-step plans for practical research

Step-by-step plan 1 – preparing Petri dishes:

1. Add 2.0 mass-% agar and 0.33 mass-% carrageenan to water
2. Heat the mixture until it fizzes
3. Add dissolved HCl to the mixture until pH = 5-6
4. When the mixture has cooled, fill the Petri dishes halfway

Step-by-step plan 2 – placing *P. polycephalum* in sclerotium form in Petri dishes:

1. Place a piece of sclerotium of about 0.5 cm^2 in a Petri dish with the sclerotium side down
2. Press and moisten the filter paper with one or two drops of demineralised water
3. Wrap the Petri dishes with aluminium foil
4. Add a few oat flakes a few days later, once plasmodium has formed

Step-by-step plan 3 – cutting out the maze:

1. Cut out the maze with a spatula or scalpel according to the design of the maze, which is glued to the bottom of the Petri dish
2. Remove the pieces that don't belong to the maze

Step-by-step plan 4 – placing *P. polycephalum* in plasmodium form in a maze:

1. Cut out a 1 cm^2 piece of breeding ground with plasmodium on it
2. Place the plasmodium at the starting point of the maze with the plasmodium down
3. Add one oat flake at the start towards the end point and place three oat flakes at the end point
4. Place the Petri dishes in a dark room where *P. polycephalum* can be filmed

Annex 5: photos from the practical research

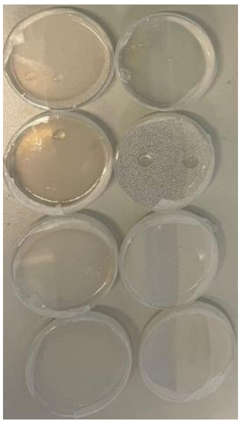

Figure 3: Eight small petri dishes with a bottom of 2.0 mass-% agar, 0.33 mass-% carrageenan and pH = 5

Figure 4: Four square petri dishes with a bottom of 2.0 mass-% agar, 0.33 mass-% carrageenan and pH = 5-6

Figure 5: P. polycephalum after adding oat flakes, 24 hours after placing sclerotium

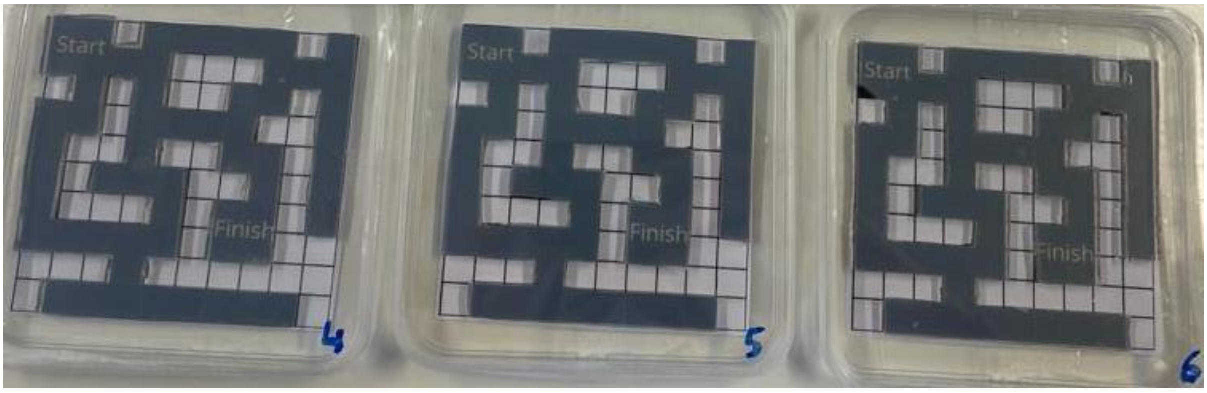

Figure 6: Petri dishes after cutting mazes

Figure 7: P. polycephalum after placing plasmodium

Figure 8: P. polycephalum after cutting 3 pieces of plasmodium for the mazes

Results maze 1:

Figure 9: P. polycephalum after placing plasmodium

Figure 10: P. polycephalum after 69 hours, has arrived at the bifurcation

Figure 11: P. polycephalum after 77 hours, goes via longest route

Figure 12: P. polycephalum after 98 hours, arriving at the finish by crossing the bottom

Results maze 5:

Figure 13: P. polycephalum after placing plasmodium

Figure 14: P. polycephalum after 101.5 hours, chooses every route, but mainly longest, contamination of oat flakes at finish

Figure 15: P. polycephalum after 107.5 hours, only continues at longest route

Figure 16: P. polycephalum after 128 hours

Figure 17: P. polycephalum reached finish after 169.5 hours, via the shortest route

Results maze 6:

Figure 18: P. polycephalum after placing plasmodium

Figure 19: P. polycephalum after 11 hours, has reached the oat flake

Figure 20: P. polycephalum after 25 hours, spreading from the oat flake in all directions

Figure 21: P. polycephalum after 36 hours, mainly goes via the two longer routes

Figure 22: P. polycephalum after 48 hours, continues on shortest route

Figure 23: P. polycephalum after 59 hours, has reached the finish via the shortest route

Results maze 11:

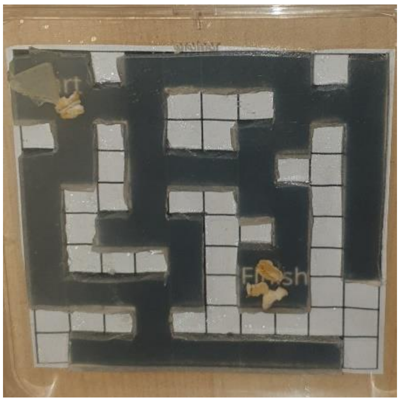

Figure 24: P. polycephalum 19 hours after plasmodium placement

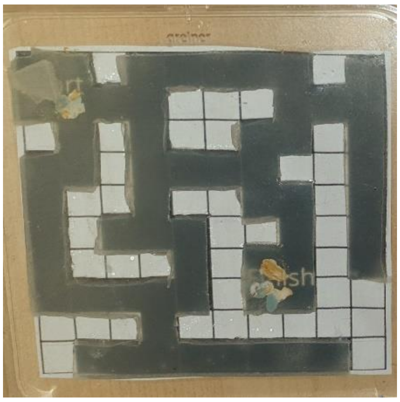

Figure 25: P. polycephalum after 76.5 hours, there is contamination at the starting oat flake, no plasmodium growth possible

Annex 6: graphs and table illustrating the results

Figure 26: distance to finish of *P. polycephalum* in Maze 5

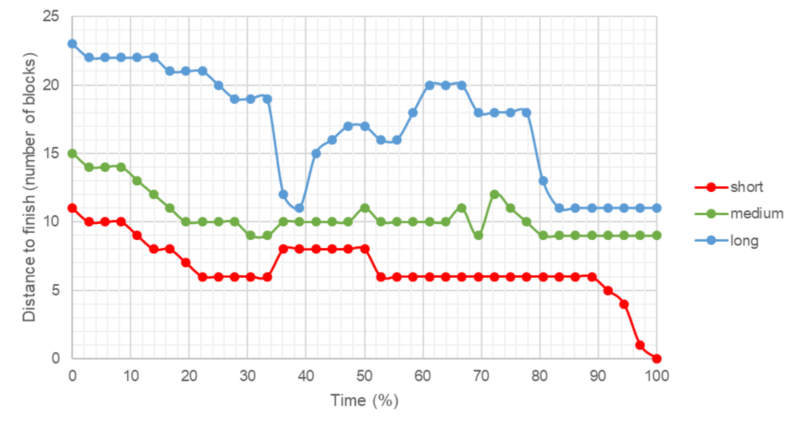

Figure 27: distance covered by *P. polycephalum* in Maze 5

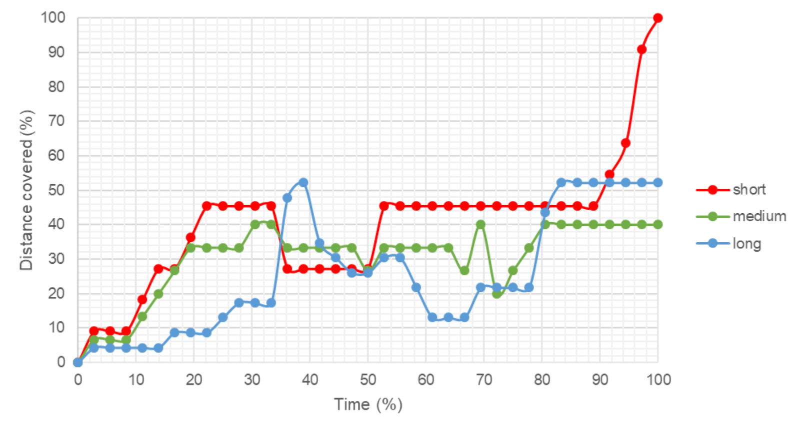

Table 1: Data on the success rate and efficiency of the growth of *P. polycephalum* in a maze

Maze	Suc-cess?	Time spent on chosen route / total time * 100%	Distance travelled via chosen route / total distance travelled * 100%	Time spent on selected route only / time spent on selected route * 100%	Time spent on chosen and different route / time spent on chosen route * 100%	Time spent on all routes at once / time spent on selected route * 100%
1	No	56	93	89	11	0,0
3	Yes	43	73	83	17	0,0
4	Yes	36	36	50	38	13
5	Yes	28	29	60	30	10
6	Yes	29	60	71	29	0,0
7	Yes	29	48	60	40	0,0
8	Yes	27	35	70	30	0,0
13	Yes	24	58	80	20	0,0
16	Yes	32	50	80	20	0,0
Average	*8/9 = 89%*	*34*	*54*	*72*	*26*	*2,5*

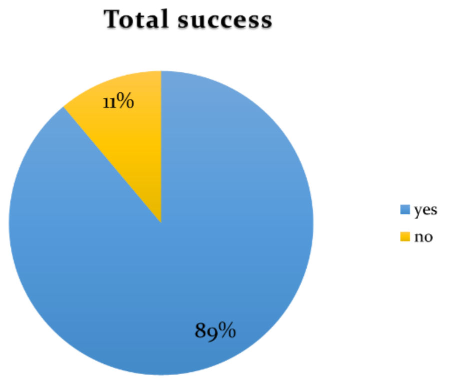

Figure 28: total success

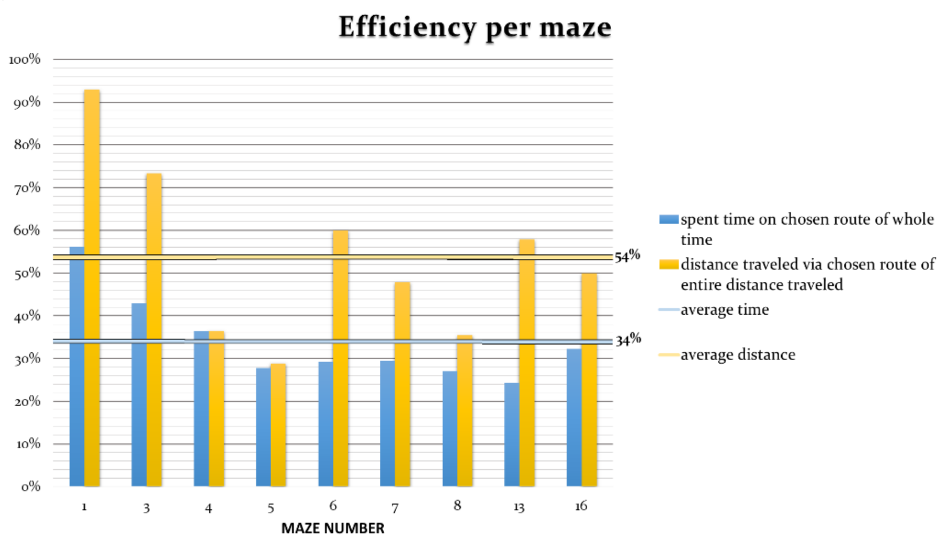

Figure 29: efficiency per maze

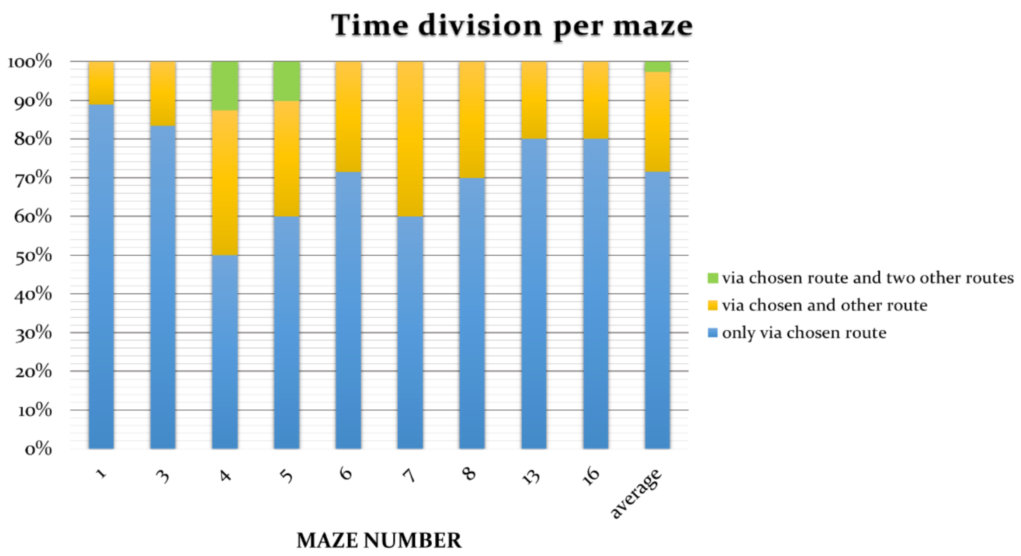

Figure 30: time division per maze