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Detection of the Genetic Relationship and Genetic Fingerprint of Real Ants (Hymenoptera: Formicidae) using RAPD-PCR Markers in Iraq

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ABSTRACT

The study aimed to investigate the molecular profiles of a number of species of real ants in some governorates of central and northern Iraq, as well as detect similarities and genetic differences based on molecular traits and determine the genetic fingerprint using RAPD-PCR indicators for 16 samples of real ants.

Samples were collected and preserved according to the methods used in preserving insects, and the DNA was extracted using a new modified method that was derived from methods used to isolate DNA from animal and plant tissues. RAPD reactions were carried out using (10) primers from the RAPD-PCR markers, and the results were carried over on a gel. The agarose bands were viewed on the UV trans eliminator, and the bands were photographed and saved until the results were studied.

The results of the RAPD-PCR markers showed different patterns of loci recognized by the primers on the genome of the samples, which is (141) loci, including one general locus for all samples and (140) divergent loci. While the primers (P3, P10) produced the least number of loci, it reached (12) loci. The total bands produced (total bands) from those loci were (720) bands, including (704) polymorphic bands and (16) main bands. P6) the least number of bands as it amounted to (46) bands, and the general variation ratio of the produced primers amounted to 99 The results of the statistical analysis showed that the values of the genetic distance ranged between (0.235 - 0.983), where the least genetic distance was between the two samples (15- *Camponotint xerxes* Salah al-Din 2) (16- *Camponotint xerxes* Salah al-Din 3), as it reached (0.235), and this is explained by the fact that there is a large similarity ratio Between the two species within the studied species, while the highest genetic dimension was between the two samples (2 - *Cataglyphis* Erbil) (16- *Camponotint xerxes* Salah al-Din 3), as it reached (0.983), and this is considered as the highest percentage of difference between the two species within the studied species, and this applies with the results of the genetic dimension of the traits appearance of the studied species.

***Keywords*-** Genetic Relationship, Genetic Variation, Real Ants, RAPD-PCR.

I. INTRODUCTION

Ants are one of the most successful groups of insects in the animal group, and they receive great attention because they form a social insect in the form of colonies or nests (dens) that sometimes contain millions of individuals. Colonies of some invasive ant species may work together to form giant colonies that extend over very large areas, and sometimes ant colonies may be described as a superorganism because they act as one coherent organism (Ward *et al*,.2005).

Ants belong to the family Formicidae, which includes about 300 genera belonging to 20 existing subfamilies, and more than 12,467,000 species worldwide (Andersen *et al*., 2003).

The molecular biology concerned with the study of insects, in terms of structure, function, and behavior, has developed greatly and amazingly over the

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past two decades, and has resulted in many surprises. With regard to genes, their structure, function and evolution, however, the application of molecular genetics to ants has revolutionized the biology of organisms and societies (Reimer, 2021).

In recent years, researchers have taken care of diagnosis at the level of molecular indicators by using DNA technologies known as genetic markers in diagnosing types of organisms, which can be applied to all stages of insect life, even to damaged or deformed samples (Al-Abbasi ,2023). The most important of these indicators are the PCR-RAPD indicators. (Random amplified polymorphic DNA), and it is one of the indicators based on PCR technology, and it was developed by the scientist Williams *et al*, (1990). The aim of the research is to uncover the genetic relationship and the genetic variation of Some types of real Ants using RAPD-PCR.

II. MATERIALS AND METHODS

Collection and preservation of samples: Samples were collected from Kirkuk Governorate, Sulaymaniyah Governorate, Salah al-Din Governorate, and Erbil Governorate during the period between 1/7/2021 to 1/10/2021 from different regions of the above-mentioned governorates, using soft forceps, as well as by hand (Kiran and Karaman, 2012).) and the samples were preserved using 70% ethanol alcohol and placed in plastic tubes marked with the date of collection and the area from which they were collected until the study was conducted (Meier, 2021).

Diagnosis of samples: The ant species mentioned in Table 1 were diagnosed at the Natural History Museum Research Center / University of Baghdad, on 4/4/2022.

Table 1: Species under study

Number	The Place of Sample Collection	Scientific Name	Number	The Place of Sample Collection	Scientific Name
1	***Camponotint xerxes***	Erbil1		Kirkuk	***Messor sp.***
2	***Cataglyphis***	Erbil		Sulaymaniyah1	***Messor sp.***
3	***Messor sp.***	Erbil		Sulaymaniyah2	***Messor sp.***
4	***Camponotint xerxes***	Erbil2		Sulaymaniyah1	***Camponotint xerxes***
5	***Messor aralocaspius***	Erbil		Sulaymaniyah2	***Camponotint xerxes***
6	***Lepisiota Formicidae***	Kirkuk		Salah Alden 1	***Camponotint xerxes***
7	***Camponotint xerxes***	Kirkuk1		Salah Alden 2	***Camponotint xerxes***
8	***Camponotint xerxes***	Kirkuk2		Salah Alden 3	***Camponotint xerxes***

DNA extraction: DNA was extracted from insects using a modified method of the first two methods mentioned by (Boyce, 1989), and the second method is (Al-Sugmiany, 2017) from (Haung, 2013).

DNA purification: The process of measuring the concentration and purity of DNA was done using a (nano drop) device, and then the sample was diluted to a concentration of 50ng / ml and preserved by freezing until use**.**

Gel electrophoresis: The necessary solutions, materials, and gels are prepared and samples loaded in the electrophoresis process according to the method mentioned by (Sambrook et al. 1989:Al-Sugmiany, 2017).

RAPD - PCR Reactions: RAPD markers were performed based on (Williams *et al*., 1990) for 16 Samples of Ants using (10) primers shown in Table (2), And the components of the reaction shown in Table (3).

Table 2: The primers used in the study

no.	**Primer**	**Sequence 5´→→→ 3´**	**no.**	**Primer**	**Sequence 5´→→→ 3´**
1.	OP A-01	CAGGCCCTTC	6.	OP C-16	CACACTCCAG
2.	OP A-06	GGTCCCTGAC	7.	OP C-10	TGTCTGGGTG
3.	OP B-04	GGACTGGAGT	8.	OP D-03	GTCGCCGTCA
4.	OP B-12	CCTTGACGCA	9.	OP D10	GGTCTACACC
5.	OP B-14	TCCGCTCTGG	10.	OP D-18	GAGAGCAAC

Table 3: Solutions used in the RAPD markers

C	Components	Volume
1	**Green Master mix**	**10µ l**

ISSN: 2583-4053
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2	**Primer**	**2 µ l**
3	**Nuclease free water**	**6µ l**
4	**DNA template**	**2µ l**
5	**Total Volume**	**20µ l**

The reaction program was applied with the pre denaturation temperature (94) for a period of (5) minutes for one cycle only, after which (38) cycle consisted of denaturation heat (94) for (45) seconds and the annealing temperature (36) for (45) seconds and the extension heat (72) For (1) minute and a final heat of extension (72) for (10) minutes, one cycle. The end of the reaction time, the tubes were removed from the thermoplastic device and kept in the freezing, and (4) microliters were withdrawn from the tubes and the mixture was loaded onto the prepared agarose gel at a concentration of 1.5% and stained with the Red say dye with the Marker volumetric guide. Then the samples were removed on the agarose gel. Then the jelly was photographed with a high-resolution digital camera and the images were saved in a computer.

Statistical analysis: The results of the multiplication operations of the primers used in the RAPD markers were taken and converted into tables, depending on the presence or absence of the DNA bands and comparing it between the different samples, where the presence of the bands is symbolized by the number (1) and the absence of the bands by the number (0), the genetic similarity coefficient was calculated As well as the genetic distance between the studied samples using Nei's factor 72 (Nei and Li. 197) The similarities and differences in the genetic material (DNA) that can be obtained from the application of RAPD-PCR markers can be adopted to determine the genetic distance, which will convert the results obtained. Which appears in the gel to characterization tables by setting (1) when the beam is present and (0) when the bands is absent.

III. RESULTS AND DISCUSSION

Results of RAPD-PCR markers: The results showed that the primers had different types of bundles as mentioned in Table (4). The total number of sites identified by the primers on the genome of the samples was (141), including one general site for all samples and (140) variant loci. The primer (P2) was characterized by the highest number. For productive loci, it amounted to (20) loci, while the primers (P3, P10) produced the least number of loci, as it amounted to (12) loci. The total bands produced (total bands) from those loci were (720) bands, including (704) polymorphic bands and (16) main bands. P6) the least number of bands as it amounted to (46) packages, and the general variation ratio of the produced primers amounted to 99%. (Singh and Achint, 2017:Bajpai and Tewari., 2010: Al-Mashhadani, 2021).

Table 4: Results of the primers used in RAPD reactions for samples

c	Primer Number	Loci number	Monomorphic loci	Polymorphic loci number	Bands number	Monomorphic bands number	Polymorphic band number	Unique bands	Absent bands	variation ratio %
1	P1(OPA-05)	15	-	15	59	-	59	2	-	100
2	P2(OP B-16)	20	-	20	90	-	90	2	-	100
3	P3(OP B-9)	12	-	12	47	-	47	2	-	100
4	P4(OP C-07)	13	-	13	58	-	58	4	-	100
5	P5(OP C-13)	14	-	14	88	-	88	1	-	100
6	P6(OP D-17)	12	-	12	46	-	46	2		100
7	P7(OP D-01)	14	-	14	111	-	111	0	1	100
8	P8(OP E-14)	16	-	16	68	-	68	4	-	100
9	P9(OP E-11)	13	-	13	81	-	81	1	-	100
10	P10(OP F-18)	12	1	11	72	16	56	1	-	91
Total		141	1	140	720	16	704	19	1	99

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Volume-2 Issue-1 || February 2023 || PP. 226-233

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The study also showed the presence of distinct bands (absent bands, unique bands) as shown in Table (5). The total distinct bands resulting from primers were (20) bands, of which (19) were unique bands, one band was absent. The sample (13) was distinguished by the highest percentage of unique beams, which amounted to (4) bands, while a number of samples obtained unique beams. As for the absent bands, the sample (2) had only one absent bands. (Singh and Achint, 2017) (Sultan and Qadir, 2015).

The molecular sizes of the produced bundles varied between (2500-100 bp), where the lowest molecular size was (100 bp), in most primers and the highest molecular size in the initiator (P5), where the molecular size was (2500 bp). The efficiency of the primers used in the study varied between the studied samples, and the highest efficiency was for the primer (P7), which amounted to (15.4), and the lowest efficiency was for the primer (P6), as it recorded (6.38). The discriminatory ability, while it reached (15.7), while the least discriminatory ability was recorded for the markers (P7), as it reached (6.5) (GLÁUCIA *et al.*,2019) (Bajpai, 2016: Brito , 2008).

Table 5: Distinctive bands the efficiency of the primers, and the discriminatory ability

N	primer name	Molecular Wight	Distinctive bands in Ants samples																The efficiency	Discriminatory ability
			2		4		5		7	9	10	13		14		15	16			
			unique	Absent	unique	Absent	unique	Absent	unique	Absent	unique	Absent	unique	Absent	unique	Absent	unique	Absent			
1	P1	300–2000bp			1												1		8.2	8.3
2	P2	400–1600bp								1							1		12.5	12.7
3	P3	200–1500									1						1		6.5	6.6
4	P4	200–1600			1				2		1								8	8.2
5	P5	300–1500									1								12.2	12.5
6	P6	100–1250					1						1						6.3	6.5
7	P7	300–1000		1									3		1				15.4	15.7
8	P8	250–1500																	9.4	9.6
9	P9	200–1500					1												11.2	11.5
10	P10	200–1500	1																10	7.9
			1	1	2		2		2	1	3		4		1		1	2		

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www.jrasb.com

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Volume-2 Issue-1 || February 2023 || PP. 226-233

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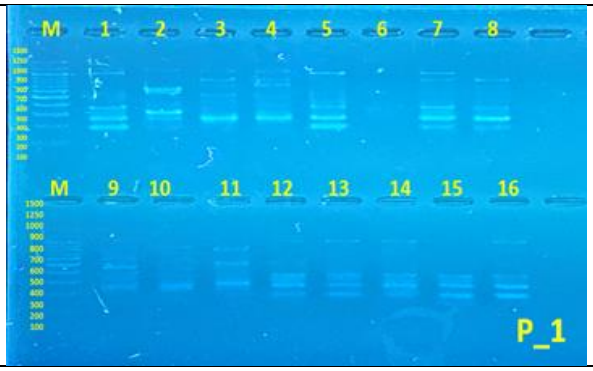
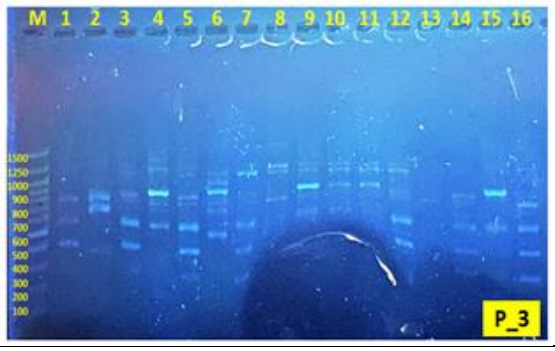
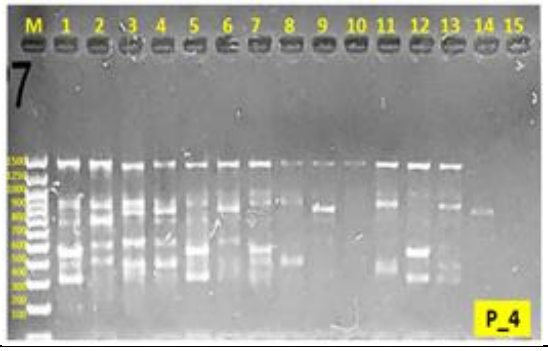
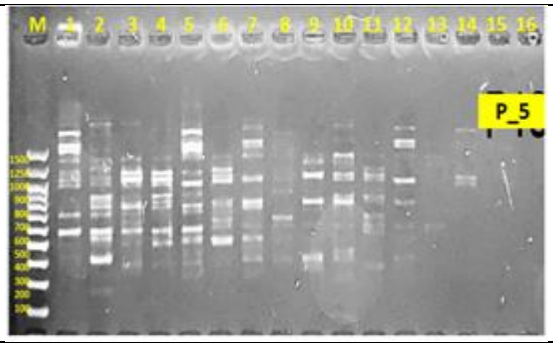


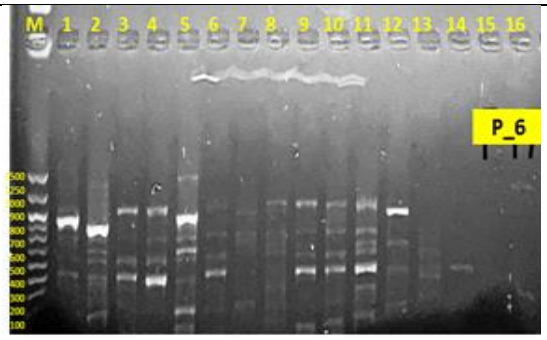
Picture (1,2) The products of the primers P1, P2 of the four types of stage flies



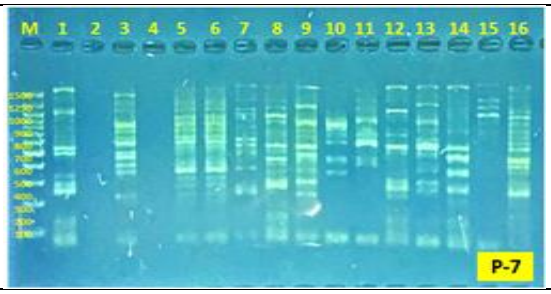


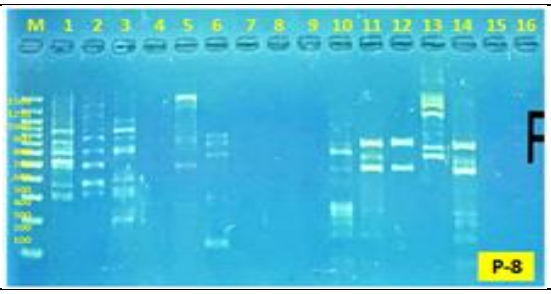
Picture (3,4) The products of the primers P3, P4 of the four types of stage flies





Picture (5,6) The products of the primers P5, P6 of the four types of stage flies





Picture (8,7) The products of the primers P8, P7 of the four types of stage flies

Journal for Research in Applied Sciences and Biotechnology

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Volume-2 Issue-1 || February 2023 || PP. 226-233

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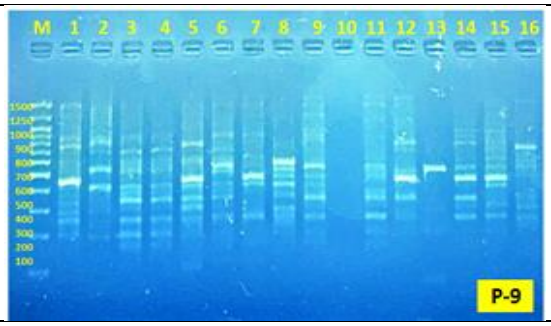

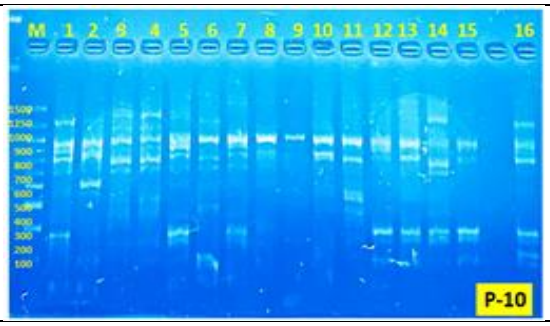

Picture (9,10) The products of the primers P9, P10 of the four types of stage flies

Estimating the genetic distance: An estimate of the genetic dimension was carried out through the results of the RAPD-PCR markers between (16) isolated samples from different regions of different types of real ants using the genetic program (NTSYS-PC.version 2.02i), which is based in its analyzes on the equation (Nei and Li, 1979). The values shown in Table (6) The values of the genetic dimension of the samples of ants studied using (10) starting from the primers of the RAPD-PCR markers. The results show that the values of the genetic distance ranged between (0.235 - 0.983), where the lowest genetic distance was between the two samples (15 and 16). It reached (0.235), and this explains that there is a large percentage of similarity between the two species within the studied species, while the highest genetic distance was between the two samples (2 and 16), as it reached (0.983), and this is considered as the highest percentage of difference between the two types within the studied species, and this applies Somewhat with the results of the genetic distance of the phenotypic traits of the studied samples. (malviya, 2011-2012-2015: Sultan and Qadir, 2015: Al-Abbasi ,2023).

Cluster analysis: Based on the values of the genetic distance of the studied species obtained from the results of the RAPD, the cluster analysis group shown in chart (1) was created, where the genetic relationship through chart (2) showed that it was divided into two main groups, group (A and B), which included Group (A) the metal fly only, and this indicates that the metal fly is more different from the rest of the species, and therefore it has the highest genetic distance within the studied species, while group (B) included the other three species, and group (B) was divided into two subgroups, which are group B1 and B2, where group (B1) included the house fly only, while group B2 was divided into two subgroups (B2a, B2b) and the group (B2a) included the meat fly, while group (B2b) included the fourth type, which is the horse fly, and this explains However, group (B2), which includes both the horse fly and the meat fly, has the least genetic distance and is therefore the most similar among the species that have been studied molecularly and this applies to what was mentioned by (Sultan and Qadir., 2015: Al-Abbasi ,2022,2023) and (malviya, 2011,2012,2015). Al-Mashhadani, 2021) On the genetic distance.

IV. CONCLUSIONS

We conclude from the above results the degree of close affinity between the Some types of ants, although they belong to different families, but they all belong to one order, which is the (Hymenoptera: Formicidae) order, and this indicates that the molecular markers, including the RAPD, can be used in the classification of insects, especially ants, in a precise classification and identification The genetic imprint has instead of adopting the phenotypic traits in the classification because the phenotypic traits are unstable and change with the change of environmental conditions in addition to the great similarity between the types of ants, especially the species, including the genus, which makes the phenotypic classification process difficult and imprecise.

Table 6: The values of the genetic dimension of Molecular Data

0.000				
0.438	**0.000**			
0.413	**0.457**	**0.000**		
0.624	**0.626**	**0.382**	**0.000**	
0.504	**0.659**	**0.659**	**0.717**	**0.000**

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Volume-2 Issue-1 || February 2023 || PP. 226-233

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0.528	**0.650**	**0.413**	**0.654**	**0.478**	**0.000**									
0.567	**0.861**	**0.592**	**0.722**	**0.472**	**0.520**	**0.000**								
0.709	**0.837**	**0.753**	**0.693**	**0.508**	**0.432**	**0.386**	**0.000**							
0.751	**0.790**	**0.685**	**0.727**	**0.743**	**0.516**	**0.614**	**0.530**	**0.000**						
0.663	**0.631**	**0.598**	**0.840**	**0.722**	**0.523**	**0.689**	**0.691**	**0.623**	**0.000**					
0.676	**0.522**	**0.581**	**0.575**	**0.585**	**0.508**	**0.668**	**0.588**	**0.543**	**0.519**	**0.000**				
0.438	**0.762**	**0.598**	**0.774**	**0.522**	**0.554**	**0.430**	**0.538**	**0.475**	**0.567**	**0.612**	**0.000**			
0.588	**0.853**	**0.710**	**0.918**	**0.564**	**0.571**	**0.609**	**0.656**	**0.637**	**0.522**	**0.628**	**0.473**	**0.000**		
0.613	**0.782**	**0.551**	**0.798**	**0.613**	**0.702**	**0.668**	**0.736**	**0.635**	**0.581**	**0.659**	**0.522**	**0.538**	**0.000**	
0.786	**0.928**	**0.714**	**0.735**	**0.731**	**0.710**	**0.629**	**0.715**	**0.498**	**0.866**	**0.777**	**0.476**	**0.621**	**0.490**	**0.000**
0.596	**0.983**	**0.685**	**0.799**	**0.599**	**0.549**	**0.532**	**0.563**	**0.447**	**0.761**	**0.883**	**0.481**	**0.472**	**0.491**	**0.235**

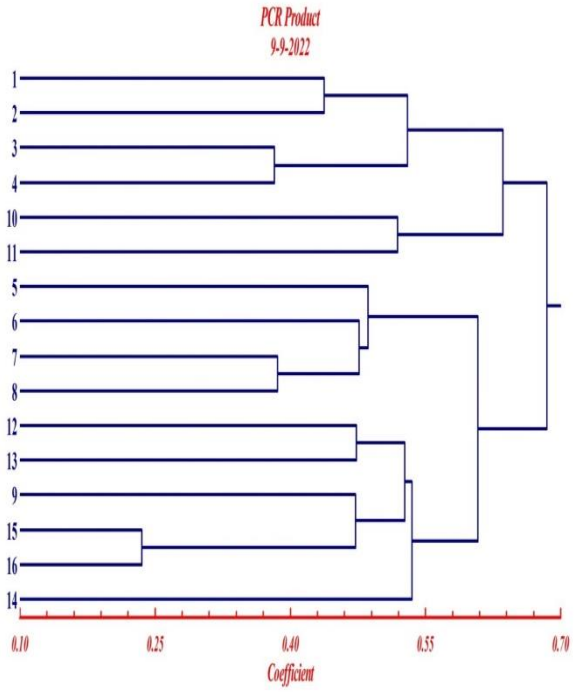

Diagram 1:Shows the genetic relationship of the four types of flies depending on the values of the genetic dimension

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