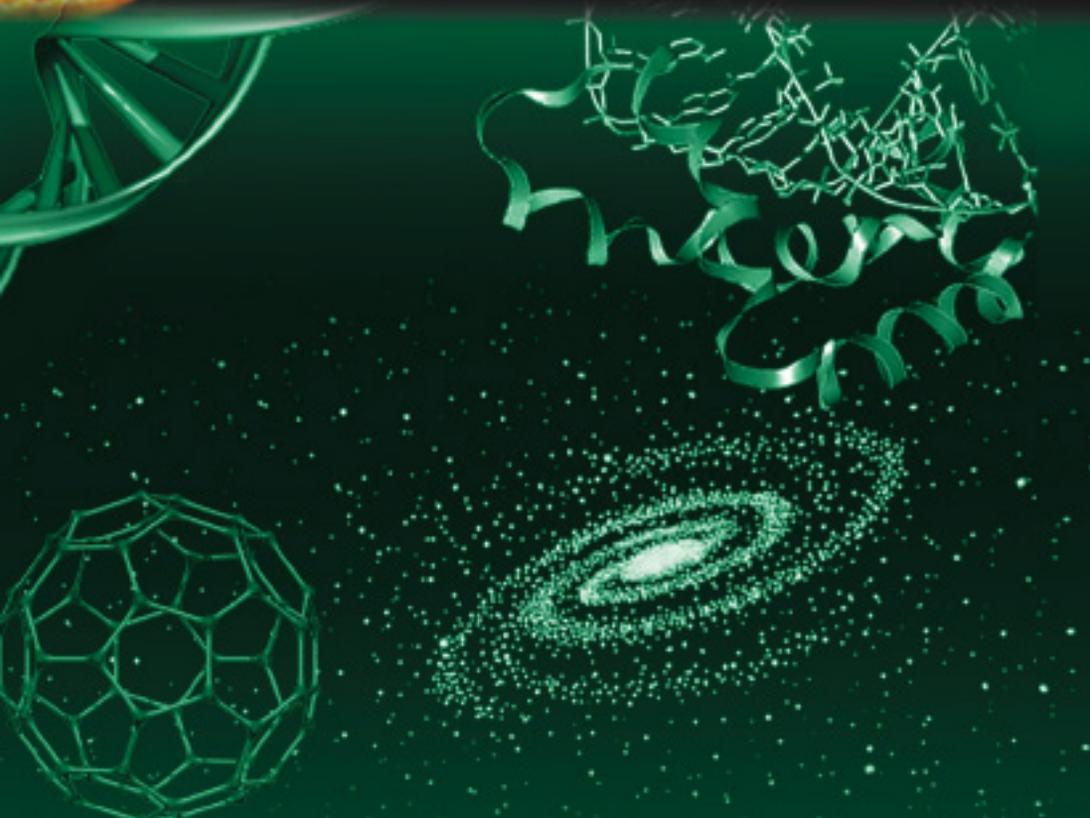


ISSN. 0377 - 2969
Vol.43(1) March 2006

PROCEEDINGS

OF THE PAKISTAN ACADEMY OF SCIENCES



*The Pakistan Academy of Sciences,
Islamabad, Pakistan*

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Published quarterly by **The Pakistan Academy of Sciences**, 3 Constitution Avenue, G-5/2, Islamabad, Pakistan.

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Website of the Academy: www.paspk.org

Proceedings of the Pakistan Academy of Sciences

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DIFFERENTIALS AND DETERMINANTS OF THE DURATION OF BREASTFEEDING IN BANGLADESH: A MULTILEVEL ANALYSIS

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Received September 2005, accepted February 2006

Communicated by Prof. Dr. M.A. Hafeez

Abstract: The differentials and determinants of breastfeeding (BF) were studied by a number of socioeconomic and demographic characteristics of mother and child in Bangladesh. The data for this study were taken from the Bangladesh Demographic and Health Survey 1996-1997. Information on BF was taken from the mothers following the last birth (n=4972) (current status) and last but one birth (n= 1851) (retrospective reporting). In this study, about 98 per cent mother in respect of last child and 92 per cent mother in respect of last-but-one child breastfed their children at least briefly. The mean duration of breastfeeding was found about 26.4 months for the last child and 25.9 months for the last-but-one child cohort. Mothers belonging to the older births cohort breastfed for a longer duration than the younger mothers. Education as well as occupation of mothers and fathers was found inversely related with the duration of BF. A positive relationship of the mean duration of breastfeeding was found with post partum amenorrhoea, age of mother at the birth of child, age of child and open birth interval.

Keywords: Postpartum amenorrhoea, open birth interval, censored, parity, proportional hazards model, relative risk.

Introduction

The benefits of breastfeeding on the health of an infant as an inexpensive and an appropriate source of nutrition are well accepted. It gives the baby best protection against diarrhoea, infections and food allergies. and thus reduces infant mortality [1]. The people who recognize the beneficial effects of breastfeeding are quite fretful about the declining trend in the duration of breastfeeding in many developing countries [2]. Several studies have identified that prolonged breastfeeding lengthens the period of non-exposure to the risk of conception and consequently increasing the interval between two consecutive births. Guz and Hobcraft [3] have showed the effect of breastfeeding beyond the start of menstruation after delivery, particularly due to the suckling stimulus as the main factor having its effect on fertility. Nath *et al.* [4] have

shown the effect of breastfeeding on the waiting time of conception after resumption of menstruation.

All of the above mentioned studies have mostly dealt with the duration of breastfeeding (full or partial) in months, but did not attempt to elucidate the level of breastfeeding. The main objectives of this paper were to study determinants of duration of breastfeeding of Bangladeshi women with a set of explanatory variables, as well as its level. This paper is divided into two parts. The first part deals with differentials and determinants of the duration of breastfeeding with some explanatory variables by using survival analysis and proportional hazard model techniques. The second part discusses any variation in the level of the duration of breastfeeding in the light of 'retrospective' as well as 'current status' reporting of the duration of the breastfeeding data.

Materials and Methods

The data for this study were taken from the Bangladesh Demographic and Health Survey (BDHS) 1996-97 [5]. A total of 4,972 women provided information on the duration of BF for their last born child, of whom 2.2 per cent reported to have never breastfed, 28.6 per cent had already weaned, 5.8 per cent experienced death of the child, and the remaining 64.9 per cent were still breastfeeding at the time of the interview. Again, a total of 1851 women provided information on the duration of BF for their last but one child (penultimate child), of whom 7.1 per cent reported to have never breastfed, 76.3 per cent had already weaned, 17.7 per cent experienced death, and the remaining 2.3 per cent were still breastfeeding at the time of the interview. It is not surprising to have some (about 2 per cent) censored cases in the case of the last-but-one child cohort also. Such reporting has also been made in several other studies based on the data from developing countries on the duration of BF [6]. Such events are usually found in cases of under nourished child and who remained sick or the mothers used to breastfeed the last-but-one child along with the last child.

The dependent variable in this study was the duration of breastfeeding. The dependent variable contained some censored cases and it was measured in completed months. The independent variables, all measured at the survey date, were grouped into three categories: demographic, socio-economic and cultural variables. Demographic variables included here were parity of mother (PARITY), current age of mother (AGEMOTH), age of mother at the birth of child (AGEMOTC), post-partum amenorrhoea (PPA), last closed birth interval (CLOSE), open birth interval (OPEN), current age of child (AGECH) and sex of child (SEX). Socio-economic variables included were education of the mother

(EDUM), education of the father (EDUF), employment status of the mother (EMPM), occupation of the father (OCUF), socioeconomic status of household (SOECOHH), and place of residence (RES). Religion (RELIG) was included as a cultural variable. The association of the duration of breastfeeding with all the explanatory variables included in this study was first checked by the Chi-square statistic. Chi-square statistic has also been used to test the goodness of fit of the model as well as to test the significance of the explanatory variables. Various summary measures based on this analysis were also calculated. Both univariate and multivariate proportional hazards model analysis were used to study the duration of breastfeeding. The univariate proportional hazard model analysis was used to give a measure of the effect of each variable on the duration-specific probabilities of the duration of breastfeeding (hazard function) in the absence of the control for other variables. A multivariate proportional hazard model analysis was then undertaken to measure the effect of each category of each variable on the hazard function while controlling the effects of the other variables (and their categories) included in the model. Some explanatory variables which were interrelated were excluded from the multivariate hazard model. A relative risk of 1.00 was considered to indicate baseline category or reference for each variable. A relative risk greater than 1.00 was taken to indicate higher risks of occurrence of the event than the reference category or vice-versa.

Figure 1 shows the distribution of the duration of BF for last and last but one child cohorts. It showed a heaping pattern in the duration of BF at the multiple of six months. A similar pattern of heaping in the duration of BF has also been reported in several other studies conducted in Bangladesh [7] as well as other developed and developing countries [6,8,9,10].

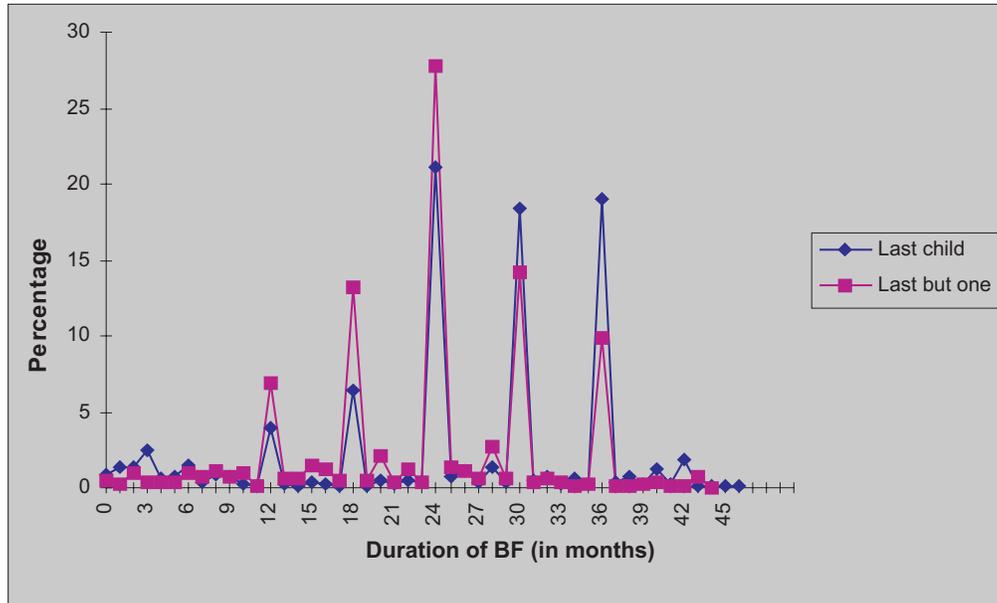


Fig. 1. Percentage distribution of duration of breast feeding.

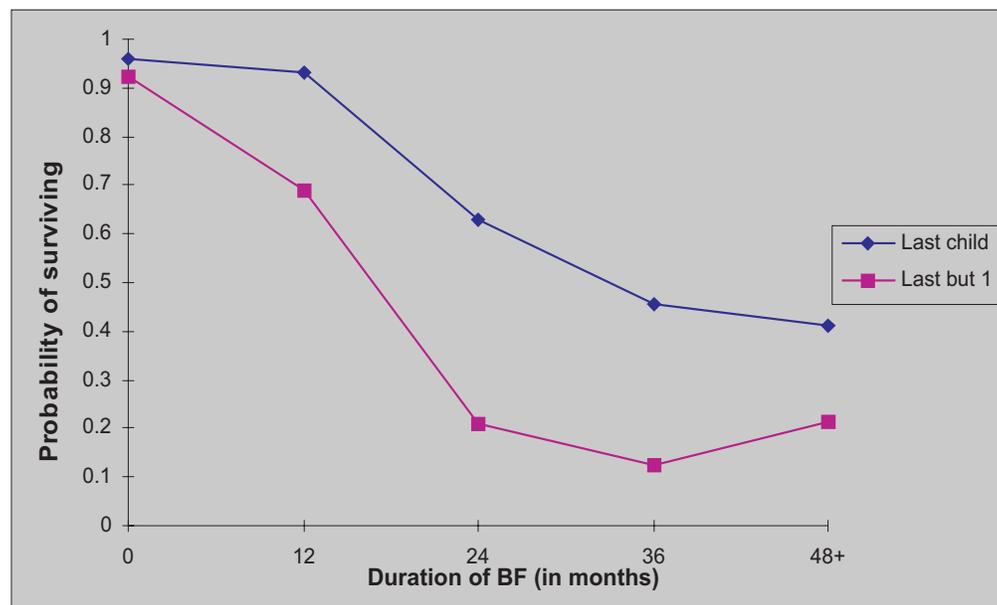


Fig. 2. Survival curves based on life table technique.

The reasons for the heaping in the duration of breastfeeding reported by these studies are: misreporting, culturally prescribed norms, memory lapse and selection bias. Nevertheless, it is difficult to detect the systematic tendencies of under reporting and over reporting in the duration of BF, unless the errors are gross. Over reporting in BF was noticed when the duration of BF exceeded the current age of the child or its age at death, whereas under reporting would not result in any visible inconsistencies and therefore remained unnoticed [6,8].

Results and Discussion

The reported mean duration of BF was 26.4 months in case of the last child and 25.9 months in case of the last-but-one child considering only the surviving children who had completed breastfeeding. The mean duration of breastfeeding found by other Bangladesh studies was between 22 to 34 months [7,11]. The present study reports a shorter mean duration of breastfeeding.

The survival analysis and its different summary measures (mean, median, trimean etc.) of the duration of breastfeeding by various characteristics of mothers and children are given in Table 1. Survival analysis, where censored cases were taken into account, revealed a higher median duration of BF (about 29.9 months) for the last child compared to the last-but-one child (about 27.0 months). This may be due to higher percentage (about 65 per cent) of the censored cases in the last child cohort than for the last-but-one child (only 2 per cent). Theoretically, survival analysis provides a higher value of probabilities at the later categories due to censoring [10]. In the present study, two survival curves of the distribution of the duration of BF for both the birth cohorts differed significantly from each other (Fig. 2).

BF in relation to parity and age

The average duration of BF in the case of the last child varied with a low value of 27.2 months for current age of the mother (<25 years) to a high value of 30.8 months for the age group 35-49 years (Table 1). The duration of breastfeeding in the case of the last-but-one child also exhibited a similar pattern where it ranged from 25.7 to 27.2 months. Further, age of the mother at the birth of the child showed an almost similar pattern in the duration of BF for the last and last-but-one child cohorts as that of the current age of the mother. The parity order of mothers also showed a similar pattern of the duration of BF as found with age variables for both the birth cohorts. For example, mothers with parity 1-2 breastfed for an average of 27.1 months, which linearly increased to 30.6 months for parity 7+ in the last child cohort. Some authors [11] have also reported a similar pattern in their studies based on Bangladesh data. This may be due to (i) mothers of high parity may be older and they produce less milk but they may be more traditional in orientation, and (ii) usually lower births occur in quick succession than higher order births, hence the chance of voluntary termination of BF at an early age of child for the younger mother might be higher than for the older mothers. Chi-square statistic revealed that parity and current age of the mother for both the birth cohorts bears a significant relationship with the distribution of the duration of breastfeeding (Table 1). After controlling the other covariates, the current age of the mother showed a significant relation with the duration of breastfeeding for both the birth cohorts (Table 3). However, parity and age of the mother at the birth of child have been excluded from the multivariate proportional hazard model analysis to avoid multicollinearity with the current age of the mother.

Table 1.
Survival analysis of BF vs other variables.

Group	Variables	No. of events	Censored cases	Proportion terminating at months			Mean	Median	Trimean	2
				12	24	36				
PARITY										
Last child	1-2	626	72.1	.0677	.3399	.5343	27.1	39.2	29.3	54.5
	3-4	429	68.1	.0593	.4209	.5455	28.1	37.1	30.6	df=24
	5-6	204	66.1	.0831	.3650	.5628	30.1	38.6	31.5	p<.01
	7+	151	64.7	.0681	.3711	.5605	30.6	38.7	32.3	
Last but one	1-2	445	2.10	.2554	.7569	.8507	26.0	27.5	26.7	35.4
	3-4	514	3.38	.2922	.7840	.8841	26.0	27.5	26.7	df=24
	5-6	249	4.96	.3388	.8063	.8846	25.7	27.0	26.1	p>.05
	7+	183	0.54	.3704	.8727	.9286	25.5	26.3	25.9	
AGEMOTH										
Last child	<25	448	79.4	.0601	.3344	.5294	27.2	39.9	28.7	35.0
	25-34	713	62.7	.0746	.3938	.5407	28.4	37.5	30.8	df=16
	35-49	247	53.0	.0657	.3827	.5779	30.8	38.1	32.8	p<.01
Last but one	<25	637	3.0	.3160	.7756	.8824	25.7	27.1	26.3	31.8
	25-34	615	3.3	.3197	.7822	.8679	25.8	27.2	26.3	df=16
	35-49	137	0.7	.2537	.8800	.8696	27.2	28.2	28.0	p<.01
AGEMOTC										
Last child	<25	825	70.7	.0664	.3719	.5583	28.0	38.3	30.2	14.7
	25-34	478	68.1	.0730	.3743	.5346	28.6	38.3	30.7	df=16
	35-49	107	64.7	.0499	.3624	.5082	31.1	39.7	33.1	p>.05
Last but one	<25	990	2.8	.3076	.7723	.8623	25.9	27.3	26.5	28.1
	25-34	350	3.9	.3486	.8194	.9118	25.4	26.6	25.9	df=16
	35-49	51	0.0	.1569	.9070	1.000	29.1	29.4	29.4	p<.05
PPA										
Last child	0-2	378	61.2	.0911	.3742	.5773	25.5	36.6	26.8	162.4
	3-5	141	68.9	.0620	.3174	.5373	26.5	39.6	28.4	df=56
	6-8	139	64.4	.0976	.4375	.4842	26.9	35.5	26.1	p<.01
	9-11	62	76.5	.0581	.4211	.4878	28.0	37.6	29.3	
	12-14	273	58.0	.0705	.3662	.5428	30.6	39.0	32.3	
	15-17	23	74.2	.0704	.3714	.6667	30.0	38.6	30.0	
	18+	375	47.5	.0445	.3786	.5521	31.5	39.0	33.1	
Last but one	0-2	327	3.8	.3794	.7692	.8684	24.2	25.5	24.6	152.8
	3-5	139	1.4	.3415	.7826	.8824	24.5	26.0	25.2	df=56
	6-8	147	2.7	.3911	.8466	.8182	23.9	25.3	24.6	p<.01
	9-11	99	0.0	.4239	.8868	1.000	24.3	24.9	24.6	
	12-14	253	1.9	.3133	.7918	.8529	26.8	27.7	27.1	
	15-17	59	1.7	.2609	.8810	1.000	27.2	28.1	27.8	
	18+	298	2.3	.1297	.7535	.8739	29.6	30.4	30.2	

Table 1 (continued)

Group	Variables	No. of events	Censored cases	Proportion terminating at months			Mean	Median	Trimean	2
				12	24	36				
CLOSE										
Last child	0-11	8	71.4	.000	.3448	.4444	30.0	41.5	31.2	
	12-23	175	65.9	.0965	.3826	.5874	28.8	37.4	29.7	64.7
	24-35	320	67.5	.0655	.3995	.6263	30.4	37.7	32.0	df=32
	36-47	257	67.9	.0681	.3876	.5502	29.4	38.1	30.9	p<.01
	48+	650	71.6	.0616	.3492	.4958	27.1	39.2	29.4	
Last but one	0-11	6	0.0	.4000	.6667	1.000	24.0	24.0	24.8	32.5
	12-23	206	2.8	.3848	.7769	.8235	25.1	26.0	25.3	df=32
	24-35	324	2.7	.3562	.8410	.9655	25.3	26.4	25.7	p>.05
	36-47	238	2.1	.2611	.8097	.8852	26.8	28.1	27.5	
	48+	617	3.4	.2837	.7628	.8556	26.1	27.7	26.8	
OPEN										
Last child	0-11	16	98.6	1.000			6.8	17.9	6.4	450.2
	12-23	46	95.6	.0365			13.6	12+	10.2	df=32
	24-35	224	75.6	.0633	.2759	1.000	23.6	38.8	25.1	p<.01
	36-47	395	39.4	.0740	.4010	.4018	29.4	37.6	30.3	
	48+	729	11.1	.0867	.4144	.6192	31.2	35.9	33.6	
Last but one	0-11	0								173.3
	12-23	28	31.7	.7910			14.1	17.3	14.8	df=24
	24-35	168	7.2	.5644	.8682	1.000	21.0	21.5	21.0	p<.01
	36-47	427	2.3	.3235	.8759	.8276	25.2	26.8	25.9	
	48+	767	28.6	.2324	.7387	.8828	27.8	28.9	28.6	
AGECH										
Last child	<1	NIL								530.8
	1-2	45	95.7	.9622	1.0		11.1	24.0+	10.7	df=40
	2-3	209	77.1	.9347	.7361		22.9	24.0+	24.4	p<.01
	3-4	391	41.6	.9297	.5883	.6148	29.2	37.4	30.1	
	4-5	439	15.9	.9317	.6156	.3663	31.8	37.1	34.2	
	5+	312	4.9	.8856	.5535	.4000	30.5	34.6	34.3	
Last but one	<1									
	1-2	21	32.3	.7222			13.4	17.4	13.9	
	2-3	161	7.5	.3974	.1171		20.3	20.8	20.2	207.39
	3-4	415	2.1	.6718	.1098	.1837	25.1	26.7	25.8	df=32
	4-5	459	1.3	.7413	.2305	.0884	27.1	28.3	27.7	p<.01
5+	335	0.3	.8006	.3083	.1410	28.7	29.8	29.8		

Table 1 (continued)

Group	Variables	No. of events	Censored cases	Proportion terminating at months			Mean	Median	Trimean	2
				12	24	36				
	SEX									
Last child	Male	720	69.3	.9357	.6287	.4467	28.23	38.4	30.6	5.2
	Female	690	69.6	.9294	.6275	.4630	28.65	38.5	30.8	df=8 p>.05
Last but one	Male	666	3.9	.6981	.2251	.1585	26.05	27.5	26.7	2.4
	Female	725	2.0	.6779	.1952	.0888	25.71	27.0	26.3	df=8 p>.05
	EDUM									
Last child	Illiterate	707	72.5	.0539	.3500	.5287	30.18	39.9	32.2	125.8
	Primary	404	67.5	.0744	.3779	.5768	29.26	38.1	31.2	df=24
	Mid-High	230	62.9	.0871	.4095	.5584	24.89	35.6	26.1	p<.01
	Inter+	69	56.1	.1795	.5977	.3636	18.52	28.8	18.4	
Last but one	Illiterate	886	3.0	.3054	.7890	.9177	26.3	27.5	26.8	31.0
	Primary	339	2.3	.3173	.8085	.7895	25.1	26.8	26.0	df=24
	Mid-High	145	4.0	.3214	.7487	.8372	25.9	27.3	26.4	p>.05
	Inter+	21	4.5	.5000	.8750	.0000	18.6	19.5	18.8	
	EDUF									
Last child	Illiterate	578	73.1	.0484	.3460	.5567	30.5	39.9	33.3	120.0
	Primary	344	70.1	.0827	.3634	.5325	29.3	38.8	31.0	df=24
	Mid-High	317	64.3	.0753	.4062	.5303	27.3	36.6	27.0	p<.01
	Inter+	162	58.6	.1208	.4819	.6286	21.8	32.2	22.8	
Last but one	Illiterate	711	3.0	.3186	.8114	.9000	25.9	27.2	26.4	36.0
	Primary	372	2.1	.3068	.7966	.8421	25.8	27.2	26.5	df=24
	Mid-High	219	3.52	.2734	.7157	.9231	26.9	28.2	27.4	p>.05
	Inter+	78	3.7	.4000	.7952	.6667	23.0	24.5	23.7	
	EMPM									
Last child	Unemployed	874	71.1	.0724	.3824	.5758	28.1	37.9	29.9	30.2
	Employed without cash	193	68.8	.0718	.4204	.5584	29.0	36.9	30.6	df=16 p<.05
	Employed with cash	343	64.9	.0515	.3219	.4831	29.0	40.6	32.3	
Last but one	Unemployed	919	3.7	.3073	.7763	.8880	26.1	27.4	26.6	15.7
	Employed without cash	203	2.4	.3033	.7985	.8235	25.9	27.4	26.6	df=16 p>.05
	Employed with cash	266	0.8	.3337	.8349	.8627	25.0	26.4	25.8	

Table 1 (continued)

Group	Variables	No. of events	Censored cases	Proportion terminating at months			Mean	Median	Trimean	2
				12	24	36				
	OCUF									
Last child	Agg.& related work.	489	71.7	.0489	.3176	.5214	30.4	40.8	32.9	85.4
	Daily labourer	461	69.3	.0707	.4189	.5697	28.2	36.8	30.0	df=32
	Service.	78	61.0	.0748	.5655	.4571	30.7	32.3	25.1	p<.01
	Business	342	66.6	.0989	.3745	.5435	26.8	37.3	28.1	
	Others	35	74.5	.0274	.3368	.8235	32.1	39.0	33.5	
Last but one	Agg.& related work.	519	2.3	.2976	.8006	.9077	26.3	27.6	26.9	38.7
	Daily labourer.	475	3.5	.2873	.7822	.8976	26.1	27.6	26.8	df=32
	Service.	52	1.9	.3696	.8070	1.000	24.0	25.3	24.5	p>.05
	Business	294	3.9	.3705	.7919	.7761	24.8	26.0	25.3	
	Others	43	0.0	.3171	.6786	.7778	27.1	28.1	27.8	
	SOECOHH									
Last child	Low	404	74.8	.0521	.3550	.5200	30.1	39.9	32.8	97.7
	Middle	818	68.4	.0721	.3742	.5462	28.9	38.4	31.0	df=16
	High	183	54.4	.1053	.4421	.6154	22.5	32.9	23.7	p<.01
Last but one	Low	597	2.3	.3237	.8073	.9000	26.1	27.2	26.6	38.8
	Middle	711	2.9	.2915	.7717	.8856	26.1	27.6	26.8	df=16
	High	75	7.4	.3857	.8434	.4444	22.5	24.8	23.3	p<.01
	RES									
Last child	Urban	254	61.46	.1658	.6273	.8333	24.9	35.9	26.2	57.4
	Rural	1156	70.80	.1481	.6143	.8410	29.2	38.8	31.2	df=8
										p<.01
Last but one	Urban	149	3.87	.3731	.6228	.7541	24.3	25.5	24.7	36.7
	Rural	1142	2.82	.3054	.8074	.9003	26.1	27.4	25.63	df=8
										p<.01
	RELIG									
Last child	Muslim	1280	69.4	.9312	.6108	.4478	28.4	37.8	30.5	36.1
	Hindu	126	70.1	.9440	.7816	.5057	29.2	43.0	33.1	df=16
	Others	4	63.64	1.000	.7500	.2000	30.0	39.8	31.5	p<.05
Last but one	Muslim	1282	2.7	.3097	.7983	.8910	25.5	27.3	26.5	9.3
	Hindu	105	6.3	.3465	.6923	.7368	25.9	26.9	27.8	df=16
	Others	4	0.0	.2500	.6667	1.000	30.0	30.0	30.0	p>.05
Total	Last child	1410	68.5	.0674	.3719	.5452	28.4	38.4	30.7	
Total	Last but one	1391	2.8	.3123	.7902	.8750	25.9	27.2	26.5	

Note : Total event case is not equal to 1410 (for last child) or 1391 (for last but one child) due to missing value

BF in relation to post-partum amenorrhoea (PPA)

A significant positive association was found between the duration of BF and post-partum amenorrhoea (Tables 1 and 3). The mean duration of BF for the different duration groups of post partum amenorrhoea increased from a low value of 25.5 months (amenorrhoeic for 0-2 months) to a high value of 31.5 months (amenorrhoeic for 18+ months) for the last child cohort, whereas it varied from 23.9 months (amenorrhoeic for 6-8 months) to 29.6 months (amenorrhoeic for 18+ months) for the last but one child. Survival analysis showed that about 17 per cent and 11 per cent of the mothers in the last and last-but-one child cohort, respectively, terminated BF during the first twelve months after experiencing 0-2 months of post-partum amenorrhoea, whereas only about 7 per cent and 4 per cent of the mothers did so for the last and last-but-one child cohort, respectively, who were amenorrhoeic for 18+ months. Univariate proportional hazard analysis also showed a significant impact of the duration of PPA on the hazard function of the duration of BF in respect of the last-but-one child (Table 2).

BF in relation to birth interval

This study showed that mean duration of BF varied according to closed birth interval from a low value of 27.1 months for CLOSE category 48+ months to 30.0 months for CLOSE category of 0-11 months. The duration of BF also varied according to open birth interval from a low value of 6.8 months for OPEN category 0-11 months to a high value of 31.2 months for the OPEN category 48+ months (in case of last child) (Table 1). In an Indian case study, an average duration of BF was found between 14.6 months (for CLOSE category of 0-23 months) to 23.7 months (for CLOSE category of 48+ months); and between 18.8 months (for OPEN category of 0-11 months) to 25.5 months (for OPEN category of 48+ months) [6]. Thus the duration of BF was found significantly related with open birth interval (Table 2). A significant relationship was maintained between these two even after controlling the effects for other covariates (multivariate hazard analysis) (Table 3). Due to linear dependency of open birth interval with age of child, it was dropped from the multivariate analysis.

Table 2.
Univariate analysis of the risks of weaning of breastfeeding on selected variables.

Variables	Last child (n=4682, censored=99)				Last but one child (n=1384, censored=41)			
	-2log L with covariates	Model χ^2	df	p	-2log L with covariates	Model χ^2	df	p
PARITY	20170.390	0.008	1	0.930	17990.51	4.454	1	0.035
AGEMOTH	20132.299	5.560	1	0.018	17965.42	0.110	1	0.741
AGEMOTC	20169.300	1.100	1	0.029	17994.17	0.865	1	0.352
EDUM	20116.450	57.085	1	0.000	17994.90	0.127	1	0.721
EDUF	19965.392	49.474	1	0.000	17824.502	0.275	1	0.600
OCUF	20088.901	12.980	1	0.000	17873.386	0.740	1	0.390
PPA	20147.159	23.213	1	0.000	17977.713	17.344	1	0.000
CLOSE	20168.660	1.751	1	0.186	17991.240	3.817	1	0.051
OPEN	20055.395	110.830	1	0.000	17902.330	83.578	1	0.000
AGECH	20051.815	119.701	1	0.000	17913.073	83.079	1	0.000
SEX	20170.238	0.164	1	0.686	17993.050	1.972	1	0.160
RELIG	20160.897	8.842	1	0.003	17993.994	0.998	1	0.031
RES	20153.524	17.994	1	0.000	17994.115	0.889	1	0.000
SOECOHH	20046.686	38.125	1	0.000	17668.222	0.0569	1	0.811

BF in relation to age and sex of child

The mean duration of BF increased with increase in age of the child in respect of the last and the last-but-one child cohorts (Table 1). Even after controlling the effects for other explanatory variables, a significant positive relationship existed between these two variables (Tables 2 and 3). For last child cohort, the mean duration of BF for the male child was slightly lower (28.2 months) compared to that for the female child (28.7 months), whereas in the case of the last-but-one child, it was higher (26.0 months) in the case of a male child compared to a female child (25.7 months). However, both cohorts showed no evidence of marked differentials in the duration of BF by sex of the child, after controlling the effects of other covariates (Table 3).

BF in relation to education and occupation variables

Education and occupation have been considered to measure socio-economic status of a woman [6]. In Bangladesh, about half of the mothers were found illiterate with no earning source. So, father's (husband's) education and occupation have also been included for this analysis. Table 1 shows that in the case of the last child mothers, having no schooling, were found to breastfeed on an average for 30.2 months which linearly decreased to 18.5 months for the mothers with higher education (Inter+). Husband's education also showed an inverse relationship with the duration of BF. A similar pattern was also observed in the case of the last-but-one child cohort. Similar findings have also been reported by other researchers based on the data from developing countries [9,12]. Univariate proportional hazard model analysis exhibited a significant contribution of education in explaining the duration of the BF in the case of

the last child cohort (Table 2). After controlling the effect of other covariates (Table 3), multivariate proportional hazard model analysis also confirmed this relation, where risk of early weaning significantly increased with increased education of mothers as compared to the illiterate mothers. This may be due to the fact that illiterate mothers do not get enough time to give breast milk and most of the educated mothers work outside in day time and thus tend to lactate for shorter period and probably also provide food supplements to the children much earlier. Husbands' education was dropped from the multivariate analysis to avoid multicollinearity problem. Women whose husbands were agriculturists or doing related works experienced a longer mean duration of BF than those women whose husbands belonged to other categories of occupation (Tables 1 and 3).

BF in relation to place of residence and socioeconomic status of household

As may be expected, rural mothers showed a longer mean duration of BF than the urban mothers for both the birth cohorts, and the relationship between these two was found significant (Tables 1 and 2). Proportional hazard model analysis (Table 3) showed that the risk of weaning of the duration of breastfeeding was lower for the rural mothers as compared to the urban mothers after controlling the effect of other variables.

The mean duration of BF was higher among mother's belonging to low socioeconomic category of households and lower for high category of households. Households of low socioeconomic status exhibited that only 7% and 6% of the mothers terminated BF at the first 12 months for the last and last-but-one birth cohort, respectively. However, their specific contribution in explaining the duration of BF was not

Table 3.
Proportional hazard model analysis: Risks of weaning of breastfeeding.

Variable	Group	Last child		Last but one	
		Risk ratio	p	Risk ratio	p
PARITY	1-2	-	-	-	-
	3-4	1.001	0.984	0.945	0.283
	5-6	1.045	0.495	0.987	0.837
	7+	1.051	0.587	1.113	0.264
AGEMOTH	<25	-	-	-	-
	25-34	1.001	0.979	1.017	0.740
	35-49	1.081	0.327	1.026	0.793
AGEMOTC	<25	-	-	-	-
	25-34	0.968	0.533	1.068	0.333
	35-49	0.852	0.101	0.849	0.197
EDUM	Illiterate	-	-	-	-
	Primary	1.156	0.084	0.943	0.478
	Mid-high	1.274	0.050	0.852	0.074
	Inter+	3.245	0.000	1.396	0.077
EDUF	Illiterate	-	-	-	-
	Primary	1.017	0.849	1.092	0.136
	Mid-high	1.076	0.484	0.879	0.051
	Inter+	1.324	0.050	0.977	0.834
EPEM	With cash	-	-	-	-
	without cash	1.257	0.038	1.011	0.844
	Unemployed	1.111	0.171	0.929	0.067
OCCUF	Agg.&related work.	-	-	-	-
	Daily labourer.	1.389	0.000	0.958	0.491
	Service.	1.084	0.644	1.235	0.121
	Business	1.322	0.023	1.023	0.741
	Others	1.404	0.106	0.887	0.363
CLOSE	0-11	-	-	-	-
	12-23	1.113	0.266	0.943	0.574
	24-35	1.104	0.263	1.084	0.412
	36-47	1.074	0.429	0.882	0.223
	48+	1.005	0.953	0.906	0.364

'-' indicates reference category

significant in both the data sets after controlling the effects for the other explanatory variables (Table 3).

BF in relation to cultural variable

The variation in the duration of BF according to cultural variables has been observed in many developing countries such as Bangladesh [11], India [9] and Ghana [8]. Hindu mothers exhibited slightly longer duration than the Muslim mothers [11].

The present study also showed a slightly higher mean duration of BF among the Hindu mothers (29.2 months in respect of last child, and 25.9 months in respect of last but one child) than their Muslim counterparts (28.4 months in

respect of last child, 25.5 months in respect of last-but-one child) (Table 1). However, no significant relationship between religion and the duration of BF was observed (Table 2).

Level of the duration of BF under both 'retrospective' and 'current' status reporting of data

Some controversies have been reported regarding the level of the duration of BF obtained from retrospective and current status reporting of data [6]. Some of the researchers have argued that a significant difference of the duration of breastfeeding was obtained under these two types of data reporting. However, [10] argued that the differential does not exist whether BF data is obtained from either the retrospective or from

Table 3 (continued)

Variable	Group	Last child		Last but one	
		Risk ratio	p	Risk ratio	p
OPEN	0-23	-	-	-	-
	24-35	0.783	0.049	0.726	0.045
	36-47	0.719	0.249	0.822	0.257
	48+	0.897	0.724	1.067	0.775
AGECH	<1	-	-	-	-
	1-2	0.778	0.555	4.534	0.872
	2-3	1.143	0.624	4.720	0.868
	3-4	1.286	0.356	2.881	0.909
	4-5	1.510	0.170	1.740	0.953
	5+	1.853	0.055	1.535	0.964
SEX	Male	-	-	-	-
	Female	0.991	0.742	1.045	0.111
RELIG	Muslim	-	-	-	-
	Hindu	0.658	0.001	0.959	0.081
	Others	1.079	0.914	1.010	0.977
RES	Urban	-	-	-	-
	Rural	1.011	0.803	1.009	0.844
SOECOHH	Low	-	-	-	-
	Middle	0.976	0.589	0.976	0.638
	High	1.069	0.378	1.025	0.789

'-' indicates reference category

the current status reporting. Theoretically, the difference depends on censoring observations because survival analysis inflates probabilities at the later categories of the duration variable due to censoring. Figure 2 also exhibits a higher probability at the later categories of the duration of BF for the current status data (last child) than the retrospective data (last-but-one child).

As discussed earlier, the duration of BF (mean, median or mode) and its demographic and socioeconomic correlates were calculated from both the 'retrospective' and 'current' status data set. While the differentials in the duration of BF by characteristics of mother and child were identical in both the data sets, the median duration of BF was quite different. A higher median (29.9 months) and trimean (28.7 months) of the duration of BF was found for the last child than the last-but-one child (median 27.0 months and trimean 26.5 months). Questions also arise as to why a higher median duration of BF exists subsequent to the birth of last child than the last-but-one child and whether such a differential really exists or not between the retrospective and the current status reporting of the duration of BF? To answer these questions, the mothers were selected who had given at least two live births at the time of the survey and who had completed their BF. There were 142 such mothers for both the last child and last-but-one child cohort. For this sample, the median duration of BF obtained was 26.9 months and 27.1 months for the last and the last-but-one child cohort, respectively. Thus, no significant difference was observed in the duration of BF regardless of whether it was obtained under "retrospective" reporting or current status reporting. However, the 'current status' data may have better coverage than the censored cases, whereas the 'retrospective' reporting may have missed information for some mothers. In both types of data, the reporting of the duration of BF might be influenced by recall

lapse and memory biases. Nevertheless, the recall lapses may be higher for those births which occurred a long time ago i.e. in 'retrospective' reporting. On balance, it appears that the 'current status' data may provide some consistent estimates of the mean duration of the BF than the retrospective reporting.

In conclusion, this study shows that the breastfeeding phenomena noted above for Bangladesh are shared universally by other developing countries. According to the present investigation the duration of breastfeeding is longer for mothers of higher parity than other mothers but this difference does not reveal statistical significance. The duration of breastfeeding increases with increase in duration of post partum amenorrhoea. Education and occupation of both the mothers and the fathers was found inversely related with the duration of breastfeeding. The sex of the child shows no differentials in the duration of breastfeeding. The Hindu mothers breastfeed for a longer duration than the Muslim mothers. The study also reveals that mothers belonging to a high socioeconomic status breastfeed for a shorter duration than their socially deprived counterparts. The education of women, employment status of women and occupation of husbands has a significant negative effect on the duration of BF. The study provides an opportunity to examine the duration of BF in respect of the last child (current status) and the penultimate child (retrospective one). There is a debate in the literature as to which of these two types of data gives better estimates of the mean duration of BF. In fact, no significant difference was found in this study in the mean duration of BF regardless of whether the data come from 'retrospective' or 'current' status reporting. So far as the quality of the data is concerned, the 'current' status data may provide a better source for estimating the level of the duration of BF than the 'retrospective' reporting.

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FUNCTIONAL ASSESSMENT OF URBAN FORESTED WETLANDS

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Received November 2005, accepted January 2006

Communicated by Prof. Dr. Rafiq Ahmad

Abstract: Wetlands perform various functions of vital socio-ecological significance. To avoid further loss of functions, functional assessment techniques for management purposes are important to develop for different wetland classes. Our aim was to assess the biotic functions of urban-forested wetlands, and to evaluate specific functional assessment models in an urban setting. The models were adopted from the low gradient riverine wetlands hydrogeomorphic (HGM) functional assessment guidebook of Western Kentucky of the US Army Corps of Engineers. Three bottomland hardwood wetlands were chosen for assessment and models evaluation in East Baton Rouge Parish (EBRP), Louisiana. Fourteen out of 17 variables for nutrient cycling, maintenance of native plant community and provision of habitat for wildlife functions were applicable to the selected wetlands. Three surrogate variables were developed to fill identified gaps in the existing models and provide more accurate assessment of urban forested wetlands. Litter layer depth was found to be a more reliable assessment variable for quantifying O-horizon biomass production than the presence/absence of an O-horizon. Dominant wetlands plant species list was adjusted to accurately reflect the flora of the urban forested wetlands of EBRP. An additional variable for characterization of forest strata as a factor of wildlife habitat provision was developed, and added to the model. Overbank flood frequency variable was not applicable to the fragmented urban wetlands and was removed from the models. The amended assessment models accurately captured existing wetland conditions and the effects of site alterations due to urbanization. These alterations caused significant differences ($p < 0.05$) in wildlife habitat provision, maintenance of characteristic plants community and nutrient cycling functions among the three sites. Further work on the application of these models in similar urban forested settings in the southeastern US is recommended.

Keywords: Hydrogeomorphic functional assessment; bottomland hardwood forest; wetland assessment; Urban-forested wetlands; ecological functions

Introduction

Wetlands perform key ecosystem functions that maintain the ecological integrity of the wetland ecosystems [1]. They provide wildlife habitat, recharge ground water and deep aquifers, recycle nutrients, ameliorate downstream flooding and protect water quality and produce biomass [2,3]. The level of a function performed by a wetland is the result of its biotic and abiotic structural characteristics as well as their interactions [4].

Since European colonization, nearly 80% of the forested wetlands in the Lower Mississippi River alluvial valley have been lost due mainly to drainage and conversion to croplands [5,6]. Loss of wetland acreage means loss of specific functions that the wetland could have sustained [7,3].

The ecological importance of wetlands was recognized and protected through Section 404 of the Clean Water Act (CWA) and subsequent judicial decisions [8]. In 1980, the Federal

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Government adopted a 'no net wetlands loss' policy to ensure mitigation of wetland functions loss due to development. Under Section 404 of CWA, the US army corps of engineers (COE) is responsible for assessing impacts to wetland functions from development activities and requiring mitigation of unavoidable impacts. However, assessing wetland functions is more complicated than identifying wetland boundaries and lost acreage. Various assessment techniques have been developed since 1970's for the determination of wetland functions such as the habitat evaluation procedure (HEP), wetland evaluation techniques (WET), and hydrogeomorphic functional assessment (HGM) [9].

The COE has adopted the HGM approach for wetlands regulatory purposes [10]. The HGM approach classifies and then functionally characterizes wetlands on the basis of the hydrogeomorphic setting, water source and hydrodynamics [10]. Wetlands are classified based on these attributes and the functions supported by different wetland classes are quantified. Local and regional guidebooks have been developed that provide protocols and models for quantifying specific functions for the various wetland classes and subclasses [11]. The ability of a specific wetland to perform functions naturally varies within a wetland class. Data are collected on specific functions from a number of sites (pristine to disturbed) encompassing the natural variability within the HGM class and are called the reference wetlands. Sites among the reference wetlands which sustain a function optimally are called the reference standard wetlands [12]. The models developed for specific functions designed to result in a functional capacity index (FCI), which rank the functions on a scale from 0.00 to 1.0. FCIs indicate the capacity of wetlands to perform a function.

Each model is comprised of variables, which are analytic structural criteria of the wetlands and surrounding landscapes that influence a function. Variables are measured and ranked on a sub-index scale of 0.0 to 1.0 relative to reference standard wetlands. The sub-index values of the variables are put into the assessment model and FCIs for different functions are determined [1]. An FCI of 1.0 indicates optimal performance of a specific function comparable to that of the reference standard wetland [10].

Urbanization activities in East Baton Rouge Parish (EBRP) of Louisiana has led to the loss, fragmentation, and hydrologic isolation of forested wetlands, besides forest conversion to croplands as elsewhere in the southeast [13]. Although forested wetlands represent 33% of the total land area of the EBRP [14], they are important for flood control, nutrient cycling, sediment and metal retention [15], habitat for native and migratory wildlife species and pollution control. Applicable and feasible ecological functional assessment models are needed for these urban wetlands for making informed management decisions. Our objective was to evaluate the applicability of three biotic functional assessment models for assessing the biotic functions of urban forested wetlands of EBRP.

Materials and Methods

Research Sites

Three forested wetland sites were selected for functional assessment in EBRP. The parish is part of the Lower Mississippi River alluvial valley, which was formed by fluvial sediments deposited by Mississippi River, about 6000 years ago [16]. The alluvium is mainly comprised of montmorillonite, mica, illite, vermiculite,

feldspar, quartz and iron oxides [17]. The hardwood forests are dominated by typical bottomland hardwood tree species (*Ulmus americana*, *Quercus* species, *Acer* species, *Celtis* species). These poorly drained soils have permeability less than 5.08 cm/hour [17,18]. Two of the three sites selected for this study were located in the southern more developed section of EBRP (Burbank and Siegen sites), while the third site was located in the northern more rural part of EBRP (Flonacher site).

The Burbank site was located on 528 ha tract surrounded by roads on three sides. The major source of water at this site is precipitation and run-off from adjacent croplands. The soil of the site belongs to the Sharky series (very fine, montmorillonitic, nonacid, thermic, Vertic Haplaquepts).

The Siegen site (70 ha) is in an early secondary successional stage, after being abandoned as agricultural field. Shallow surface ditches that were dug in the past for agricultural purposes cover the site and drain into a main channel. Precipitation and run-off from adjacent uplands are the major sources of water. The soil type is Sharky series and is poorly drained [17].

The Flonacher site (24 ha) is located in the more rural northwest section of the parish. The forested wetlands of the site are flat and depressional regions surrounded by upland forests and rangelands. Precipitation, run-off from forest and rangelands and overbank flooding from a small seasonal creek are the major sources of water of the site. The soil type of the site is Sharky and Zachary series (Typic Albaqualfs). The soils are formed in silty alluvium deposited by the Mississippi River.

Assessment Models

We used the Western Kentucky functional assessment guidebook [19] to assess wetland

functions as it was the only regional guidebook available at the time of our study. The forested wetlands of EBRP originally developed under conditions similar to the low-gradient riverine forested wetlands of Western Kentucky, therefore, the models should be applicable.

Three biotic functions were selected for assessment in the three selected sites. The functions were 1) cycling of nutrients, 2) maintenance of characteristic plant community, and 3) provision of habitat to wildlife. Assessment models for each function and their underlying variables were adopted from Ainslie *et al.* [19]. Where necessary, surrogate variables were developed to fill any gaps in the existing models.

Variable values were measured onsite and by referring to published literature. After we measured the variables, their values were converted to sub-index values on a scale of 0.0 to 1.0 based on the reference data in Ainslie *et al.* [19]. The range of sub-index values represents the contribution of the value of a variable to the functional capacity for a function by a wetland [19]. The FCIs for the three functions were determined by using the following models.

a) Cycling of nutrients

$$FCI = 1/2 \left[\left(V_{TBA} + V_{SSD} + V_{GVC} \right) / 3 + \left(V_{OHOR} + V_{AHOR} + V_{WD} \right) / 3 \right] \quad (1)$$

where

- V_{TBA} = Tree basal area
- V_{SSD} = Understory vegetation density
- V_{GVC} = Ground vegetation cover
- V_{OHOR} = O-horizon biomass
- V_{AHOR} = A-horizon biomass
- V_{WD} = Woody debris biomass

b) *Maintenance of characteristic plant community*

$$FCI = \left[\frac{(V_{TBA} + V_{DEN})/2 + V_{COMP} \times \frac{(F_{SOILINT} + V_{WTD})}{2}}{2} \right]^{1/2} \quad (2)$$

where

V_{TBA} = Variable tree basal area
 V_{DEN} = Trees density
 V_{COMP} = Plant species composition
 $V_{SOILINT}$ = Soil integrity of the assessment area
 V_{WTD} = Depth to water table from soil surface

c) *Provision of habitat for wildlife*

$$FCI = \left[\frac{V_{MACRO} + ((V_{TRACT} + V_{CONNECT} + V_{CORE})/3)}{2} \times \frac{(V_{COMP} + V_{TBA} + V_{TDEN} + V_{SNAG} + V_{STRATA}) + (V_{LOG} + V_{OHR}/2)}{6} \right]^{1/2} \quad (3)$$

where

V_{MACRO} = Macro-topographic features
 V_{TRACT} = Wetland tract area
 $V_{CONNECT}$ = Percent of wetland tract connected to other suitable wildlife habitats
 V_{CORE} = Interior core area
 V_{COMP} = Vegetation composition
 V_{TBA} = Tree basal area
 V_{TDEN} = Tree density
 V_{SNAG} = Snag density
 V_{STRATA} = Vegetation strata
 V_{LOG} = Log biomass
 V_{OHR} = O-horizon biomass

Twelve circular plots of 11.3 meters radius were randomly established in the three sites for measuring the values of the variables. Variable tree basal area (V_{TBA} , $m^2 ha^{-1}$) was determined by measuring the diameter at breast height (DBH) of trees greater than 10 cm. The diameter values were converted to area by using the following formula.

$$\text{Area} = 5/6 \text{ Diameter}^2$$

Tree density (V_{DEN} , stems/ha) was determined by counting the number of trees having DBH greater than 10 cm and multiplied by 25 to convert it to stems/ha. Similarly snag density (V_{SNAG} , snags/ha) with DBH greater than 10 cm were counted in the plots. Plant species composition (V_{COMP}) for the three strata was determined for each site and ranked in descending order of dominance. The relative plant species dominance was calculated by using the 50/20 rule of the Federal Wetland Delineation Manual [20]. However, the updated dominant plant species list for EBRP was used for the purpose (Table 2). Shrub density (V_{SSD} , shrubs/ha) was determined in 3.6 meter radius subplots established in the main plots. Shrubs having a minimum height of 1 meter with DBH less than 10 cm were counted in the subplots. Percent ground vegetation cover was determined visually in four subplots (1 m^2) in the main plot. O-horizon biomass (V_{OHR}) was determined by taking leaf litter depth in 4 1- m^2 subplots in the main plot. A sub-index scale developed for EBRP was used to assign sub-index values to the variable (Fig. 3). The presence, absence and thickness of soil A-horizon (V_{AOHR}) was determined by taking 15 cm deep soil cores through a hand auger in the 1- m^2 subplots. Woody debris biomass (V_{WD} , tons/ha) in each plot was determined by counting the number of stems intersecting 2 transects of 125 cm.

The depth to seasonal high water table (V_{WTD}) was determined by digging ground water table monitoring wells in spring 2001. The depth to the ground water table was determined and averaged for the spring season in the main plots. Soil integrity ($V_{SOILINT}$) variable was determined visually on-site by looking for any soil disturbance indicators (excavation material, fill, plowing, and compaction). Absence of these indicators in the plots indicated that the soils were intact. Forest strata variable (V_{STRATA}) was determined based on the absence/presence of the forest stand stratification in the assessment plots (Table 2).

The landscape scale variables (wetland tract area (V_{TRACT}), habitat connections (V_{CON}), interior core area (V_{CORE}), and macrotopographic features (V_{MACRO})) for the three assessment sites were determined by using Louisiana GIS database maps and USGS quad maps [21]. Sub-index values determined for the 17 variables of the 12 plots for the three sites were averaged and FCIs were calculated with the assessment models (Eqns. 1, 2, and 3).

Statistical Analysis

Linear regression of litter layer depth, leaf litter weight and tree basal area was analyzed with the SAS REG procedure. One-way ANOVA was used to test for significant differences among the three sites for the three biotic functions [22]. Post-hot Fisher's protected pairwise comparisons were done for finding differences among the sites for the selected functions. The data was checked for normality of the residual assumptions of the general linear model procedures of ANOVA and regression.

Results and Discussions

Cycling of Nutrients

Out of the 6 variables of this model (Eqn.1), only the characterization technique of the O-Horizon biomass variable given in the Western Kentucky guidebook was not applicable to the wetlands of EBRP. The O-horizon is the partially decomposed organic matter and recognizable twigs and leaves overlying the A-horizon [23]. The O-horizon biomass is an active pool of nutrients in the litter layer, which decomposes and releases the bound nutrients and make them bioavailable [24]. The measurement protocol for this variable is to visually determine the percent cover of ground surface by leaf litter [19]. The generally high litterfall and moist to wet soil conditions in bottomland hardwood forests in

EBRP yield an O-horizon. Percent cover by an O-horizon was 100% for all the sites.

We developed an alternative approach for the O-horizon variable by quantifying the mass of the litter layer/O-horizon per unit area and determining whether this variable could be accurately represented by depth of the litter layer/O-horizon. The dry weight of the litter layer/O-horizon was determined in four randomly selected 1-m² subplots within each main plot. Average depth of the litter layer/O-horizon was recorded for each subplot before collection. Average weight, depth, and tree basal areas for the three research sites are shown in Table 1. Linear regression of the average litter layer/O-horizon depth on litter/O-horizon weight identified a significant linear relationship (Fig. 1) between the litter layer/O-horizon depth and weight ($r^2 = 0.50$, $p < 0.01$). The linear relationship of litter layer/O-horizon mass and depth captured the assessment sites condition. Assessment plots with high mass of litter layer/O-horizon had a high tree basal area (TBA) than plots of low litter layer/O-horizon mass. A linear regression (Fig. 2) identified a significant relationship of TBA and litter layer/O-horizon weight ($p < 0.01$ and r^2 of 0.51). Research sites in EBRP with TBA of 15 m²/ha or more had a litter layer biomass of more than 500g m⁻², which indicated optimally functional sites. This result suggested that litter layer/O-horizon depth accurately represented O-horizon biomass variable.

Table 1.

Average litter layer weight and depth of urban-forested wetlands in EBRP, Louisiana (\pm values are standard errors of the mean).

Research plots	Mean litter layer weight (g/m ²)	Mean litter layer depth (cm)	Mean Tree Basal Area (m ² /ha)
Burbank Site	513 \pm 44.2	2.20 \pm 0.13	17.1 \pm 0.50
Flonacher site	626 \pm 110	2.51 \pm 0.33	24.1 \pm 10.1
Siegen Site	422 \pm 30.1	2.11 \pm 0.42	7.0 \pm 1.45

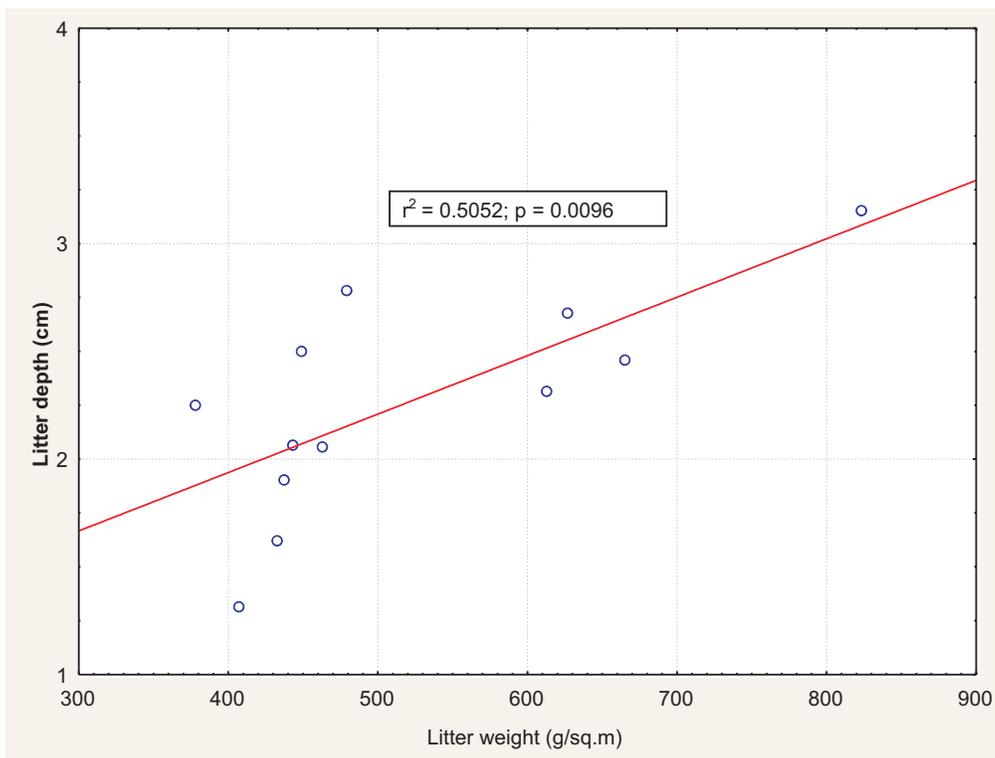


Figure 1. Linear regression graph of litter layer depth on litter layer weight of urban forested wetlands of EBRP, Louisiana.

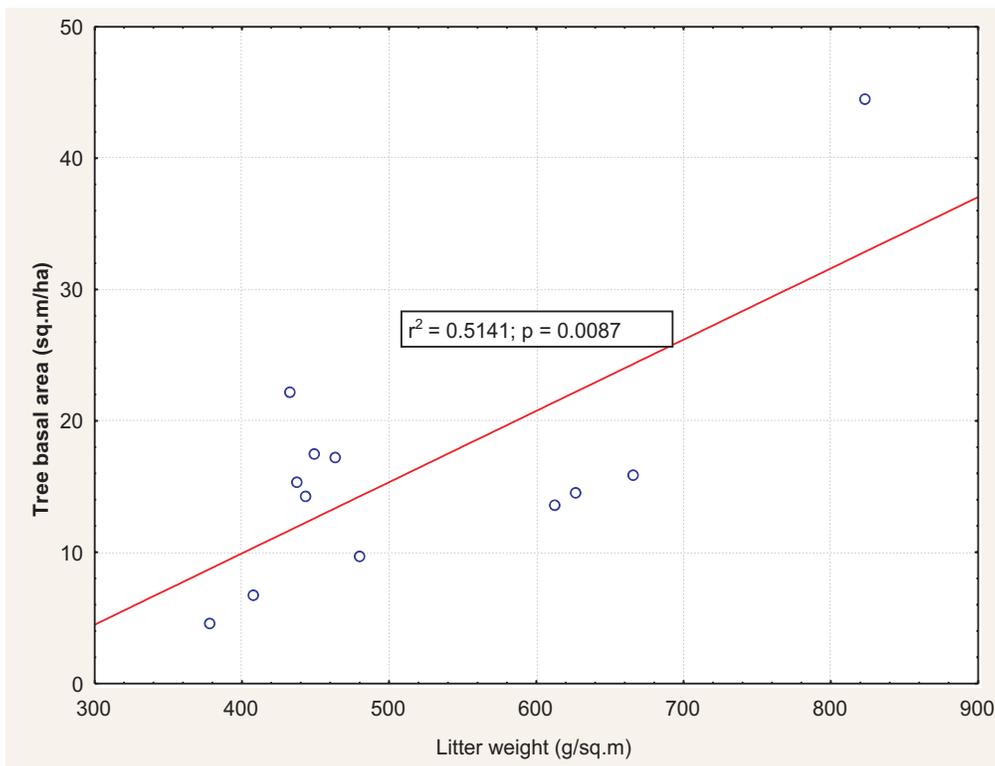


Figure 2. Regression graph of tree basal area on leaf litter weight of urban forested wetlands of EBRP, Louisiana.

Average litter layer/O-horizon mass for all the three sites before the autumn litter fall was 519 g m^{-2} . Litter layer/O-horizon production per year at the end of the autumn leaf fall would be higher than the recorded value. Litter fall production of bottomland hardwood forests of the southeast is $574 \text{ g m}^{-2} \text{ y}^{-1}$, while for naturally flooded swamps the value is $418 \text{ g m}^{-2} \text{ y}^{-1}$ [25, 26]. For periodically flooded riverine forest systems in Louisiana, a leaf litter production of $725 \text{ g m}^{-2} \text{ y}^{-1}$ has been reported [27]. Similarly, naturally flooded swamps were found with $630 \text{ g m}^{-2} \text{ y}^{-1}$ of leaf litter production in Southwestern Louisiana [28]. Leaf litter accounts for 41% of the net primary productivity in bottomland hardwood forests [25]. The litter layer/O-horizon biomass determined in this study is comparable to that reported by Conner [25], Megonigal [27] and Hoepfner [28] for bottomland hardwood forests in the Southeast.

Thus litter layer/O-horizon mass of 500 g m^{-2} or more indicates an optimally functioning site regarding the role of O-Horizon biomass in EBRP. Based on the significant linear relationship

of litter layer/O-horizon weight and depth, depth could be used as a surrogate indicator of the O-horizon biomass production and its contribution to the nutrient cycling function. Litter layer depth as a measurement technique for the O-horizon characterization was also developed for the HGM assessment guidebook of the forested wetlands of Yazoo delta, Mississippi [29], which also falls in the Lower Mississippi alluvial valley like that of EBRP. Fig. 3 was used to scale this variable for the research sites. Litter layer/O-horizon depth may decrease in spring, yet the scaling range is large enough to (2 to 5 cm litter depth for optimally functioning sites) to account for minor seasonal changes in litter layer/O-horizon depth.

Maintenance of Characteristic Plant Community

In this model, the overbank flood frequency variable was removed from the model because of the absence of a hydrological connection between the wetlands in the EBRP and active streams or rivers.

The major sources of hydrological input into the wetlands are precipitation, and run-off from

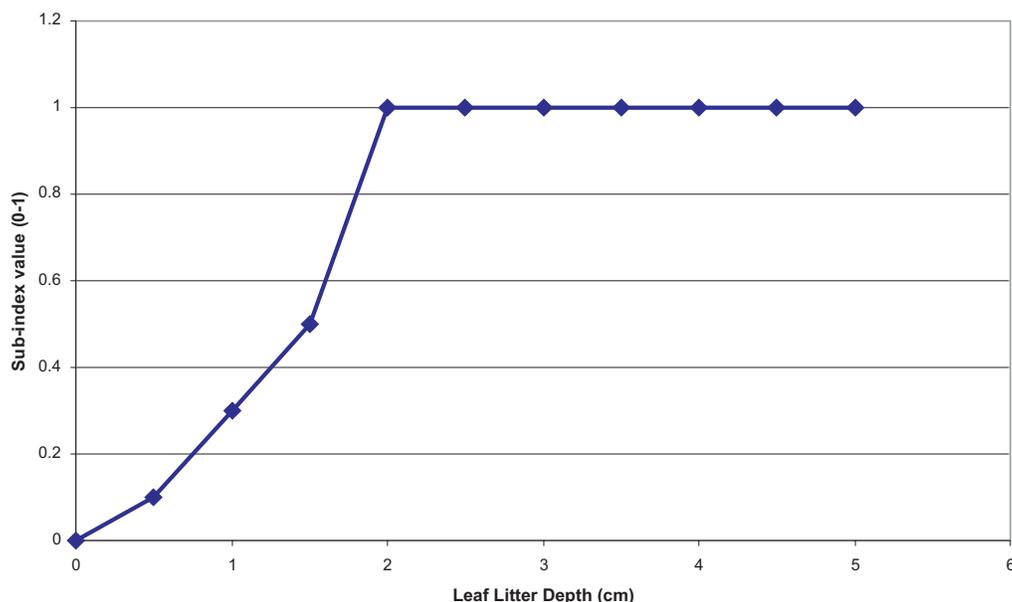


Figure 3. Litter layer/O-horizon biomass sub-index score determination graph.

roadside ditches and small creeks that carry rainfall and urban run-off. The small seasonal channels and creeks that empty into the forested wetlands are not monitored for their flow regime by any state or federal agency. Therefore, the overbank flood frequency variable was dropped from the model to make it representative of urban forested wetlands.

Plant species composition variable (V_{COMP}) of this model was adjusted for EBRP by adding and removing species from the plant list. Some of the dominant species given in the Western Kentucky guidebook do not occur as dominant species in the wetlands of the EBRP. Moreover,

Table 2.

Dominant plant species by strata in urban-forested wetlands of the EBRP, Louisiana.

Tree	Shrubs/understory	Ground vegetation
<i>Acer rubrum</i>	<i>Acer rubrum</i>	<i>Acer rubrum</i>
<i>Carya x lecontei</i>	<i>Carya x lecontei</i>	<i>Arundinaria gigantea</i>
<i>Celtis leavigata</i>	<i>Carpinus caroliniana</i>	<i>Aster sp.</i>
<i>Fraxinus pennsylvanica</i>	<i>Celtis leavigata</i>	<i>Boehmeria cylindrical</i>
<i>Liquidambar styraciflua</i>	<i>Fraxinus pennsylvanica</i>	<i>Campsis radicans</i>
<i>Quercus nigra</i>	<i>Ilex deciduas</i>	<i>Carex squarosa</i>
<i>Quercus michauxii</i>	<i>Liquidambar styraciflua</i>	<i>Eragrostis alba</i>
<i>Q.pagodaeifolia</i>	<i>Lagustrum sinense</i>	<i>Clyxeria striata</i>
<i>Quercus phellos</i>	<i>Quercus nigra</i>	<i>Hypericum sp.</i>
<i>Quercus lyrata</i>	<i>Quercus phellos</i>	<i>Impatiens capensis</i>
<i>Quercus laurifolia</i>	<i>Quercus lyrata</i>	<i>Panicum sp.</i>
<i>Salix nigra</i>	<i>Quercus laurifolia</i>	<i>Pathenocissus quiquefolia</i>
<i>Ulmus americana</i>	<i>Quercus pagodifolia</i>	<i>Pilea pumila</i>
	<i>Nyssa sylvatica</i>	<i>Quercus phellos</i>
	<i>Salix nigra</i>	<i>Sabal minor</i>
	<i>Ulmus Americana</i>	<i>Salix nigra</i>
	<i>Ilex deciduas</i>	<i>Saururus cernuus</i>
		<i>Smilacina recemosa</i>
		<i>Smilax rotundifolia</i>
		<i>Sparganium sp.</i>
		<i>Toxicodendron radicans</i>

(Plant names are according to Harrar and Harrar [33]).

some species in EBRP that occur as dominants are not listed as such in the Western Kentucky guidebook. An updated dominant plant species list developed for EBRP is shown in Table 2.

Species like water oak (*Quercus nigra*), American Elm (*Ulmus Americana L*), Laurel oak (*Quercus laurifolia Michx*), Bitter Pecan (*Carya x lecontei Little*), Dwarf Palmetto (*Sabal minor*), Chinese privet (*Ligustrum sinense*) and Red maple (*Acer rubrum L*) were found dominant in one or more than one strata of trees, shrubs or ground vegetation in the research plots. These species are reported as representative of the forested wetlands of EBRP [30,31,32]. Based on the dominance of these species in a stratum according to the 50/20 rule [19], all these species were added to the dominant species list of trees, shrubs or ground vegetation (Table 2).

River birch (*Betula nigra*), Shellbark hickory (*Carya laciniosa Schneid*), Shingle Oak (*Quercus imbricaria Michx*), Post Oak (*Q.stellata Wang*), Pin Oak (*Q.palustris Muench*) and Hackberry (*Celtis occidentalis L*) are dominant species in the forested wetlands of Western Kentucky. These species were not found during the surveys in the research sites in EBRP. The distribution of these species does not extend to EBRP [31,32,33]. Therefore these species were removed from the dominant plant species list to make the list representative of the vegetation type of the forested wetlands of EBRP.

Provision of Habitat for Wildlife

In this model (Eq. 3), the overbank flood frequency variable was not applicable to the wetlands of the EBRP and was removed from the model. Standard techniques given in the Western Kentucky guidebook [19] for the VMACRO, VTRACT, VCONNECT, VCORE, VTBA, VTDEN, VSAG and VLOG variables

were found applicable to the wetlands of EBRP. The changed measurement techniques of O-horizon and plant species composition variables discussed in the previous models (Eqs. 1 and 2) also apply to this model.

Depending on the age, hydrology and disturbance level, some forests in EBRP have three vegetation strata (Burbank site), while others lack one or more strata (Siegen and Flonacher sites). The presence or absence of vegetation strata (spatial stratification) affects the microhabitats available for wildlife. According to Lynch and Whigham's [34] study on habitat requirements of 15 birds, abundance was high in mature forests with tall canopies and well-developed herb and shrub layers. Bushman and Therres [35] studied 19 bird species in Maryland and concluded that the preferred habitat was a closed canopy, mature forest, with a mix of dense and open understory conditions. Some forest organisms are limited to a particular stratum, and differences in plant community structure and stratification between sites can lead to differences in animal diversity and composition [2]. The richness and diversity of consumer species is dependent on plant diversity that produces structural niche differentiation [36]. Understory and ground vegetation [37] including ferns and lichens are important components of a stand for a variety of taxa [38], and the quantity of ground

vegetation cover is highly correlated with richness of small mammal species [39,40]. Bird species composition is more diverse when horizontal forest structure (or even the landscape) structure is more heterogeneous [41]. Contribution of herb, shrub and tree strata in providing habitat to vertebrates is used in the HGM assessment guidebook for riverine wetlands of Northern Rocky mountains [42]. Difference in stratification is likely to influence the habitat quality; therefore, a new variable, the vegetation strata (VSTRATA) variable was added to the model (eq. 3) and a sub-index value table was scaled for the variable accordingly (Table 3).

The absence of herb layer indicates that the canopy is closed and dominated by mature trees. As a result there is not enough light reaching the forest floor to promote ground vegetation growth. Consequently, this absence does decrease the diversity of habitats (heterogeneity) in the forest, yet it is not an indicator of disturbance to vegetation. Therefore, a decrease of 0.1 in sub-index value is assumed to occur in terms of provision of habitat to wildlife. Similarly, with the absence of shrub layer, it is assumed that a sub-index value decrease of 0.3 occurs. Less than 50 stems per hectare of trees or shrubs is a very sparse density [19] to be qualified as optimally functional strata regarding the provision of habitat to wildlife [43] with reference to a reference standard wetland. If tree layer is absent, this indicates either a newly regenerating site or a damaged site. In such circumstances, the sub-index value is dropped by a factor of 0.7. If all the layers are absent, and the site has a potential for restoration, then a value of 0.1 could be assigned to the site.

Functional Capacity Indices of the Selected Sites

The cycling of nutrients function varied among the sites (Fig. 4). The variability in function among the sites was mainly affected by

Table 3.
Calibrated scale for the characterization of vegetation strata variable.

Vegetation status	Sub-index values
All layers present	1.0
Herb layer absent (<5% vegetation cover)	0.9
Shrub layer absent (<50 stems/ha)	0.7
Tree canopy absent (<50 stems/ha)	0.3
Newly regenerating site (restoration possible)	0.1
Converted sites (restoration not possible)	0.0

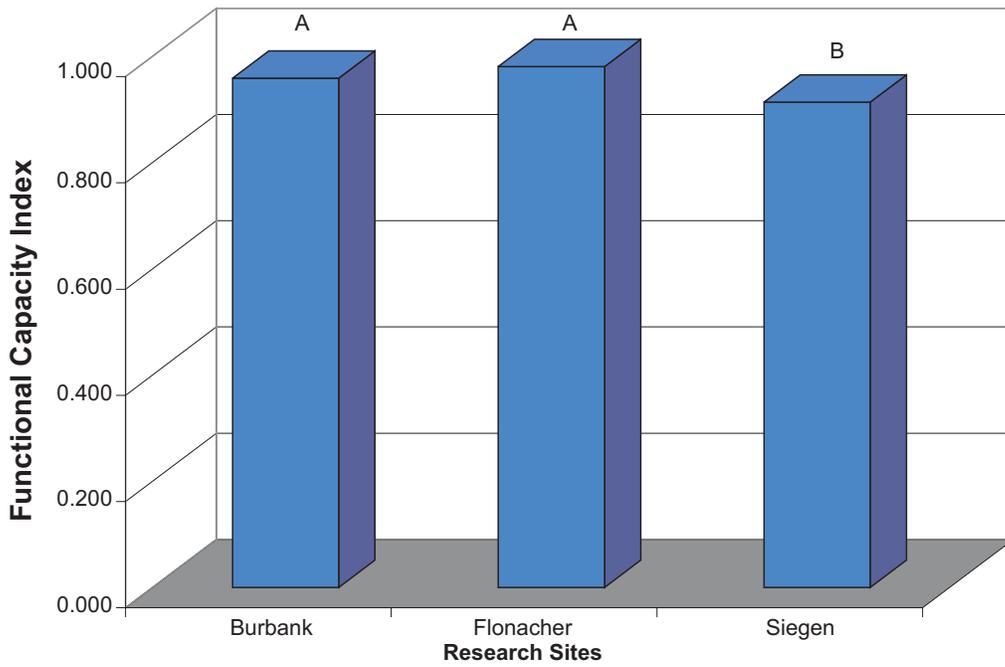


Figure 4. Mean nutrient cycling FCIs of urban forested Wetlands in EBRP, Louisiana (means with the same letters are not significantly different from each other at $p = 0.05$).

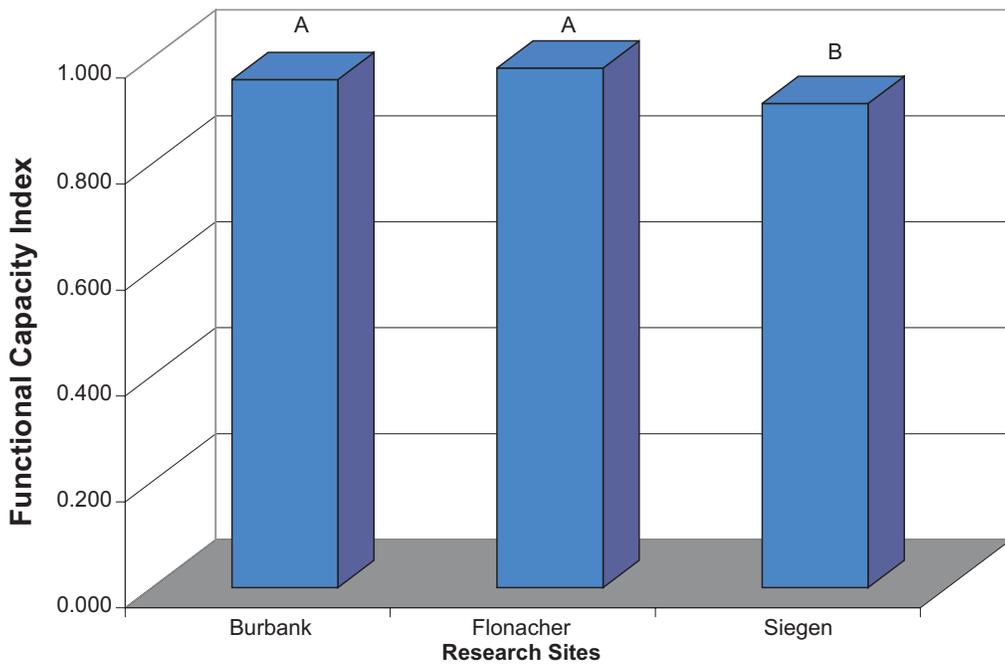


Figure 5. Mean plants habitat maintenance FCIs of urban forested wetlands in East Baton ouge Parish, Louisiana (means with the same letters are not significantly different from each other at $p = 0.05$).

the variability in the values of the biotic component of the assessment model. The FCI was 0.73 for the Burbank site, 0.68 for the Flonacher site, and 0.59 for the Siegen site. The Burbank site was significantly higher than the Siegen site ($p < 0.01$), but not the Flonacher site ($p = 0.49$).

The maintenance of the characteristic plant community function for the three sites (Fig. 5) was found to be above the 90th percentile on the functional scale. The Burbank and Flonacher sites were significantly higher than the Siegen site ($p = 0.02$ and 0.04 respectively). All the plant species reported from the three sites were facultative, facultative wet and obligate wetlands species of the region [32].

The wildlife habitat function varied significantly among the sites (Fig. 6). The function was influenced by hydrologic, landscape and biotic variables and their values were different for the three sites. The difference was

clearly reflected in the average FCIs for the three sites. The hydrologic and biotic components of the assessment model did not vary among the three assessment sites, while the landscape scale variables component varied for all the sites. The differences in the landscape scale variables led to significant functional differences in the FCIs among the three sites. The Burbank site was significantly higher than the Flonacher and Siegen sites ($p < 0.01$). The FCI of the Flonacher site was significantly higher than the Siegen site ($p < 0.01$).

Urbanization has isolated and fragmented the wetlands in EBRP, which has affected the spatial scale variables such as fragmentation, habitat connection, and interior core area. Consequently, the wildlife habitat quality FCI dropped below the 50th percentile for the three sites compared to maintenance of native plant community function (FCIs for the three sites were above the 90th percentile), which has a major role in providing wildlife habitat. Urban and

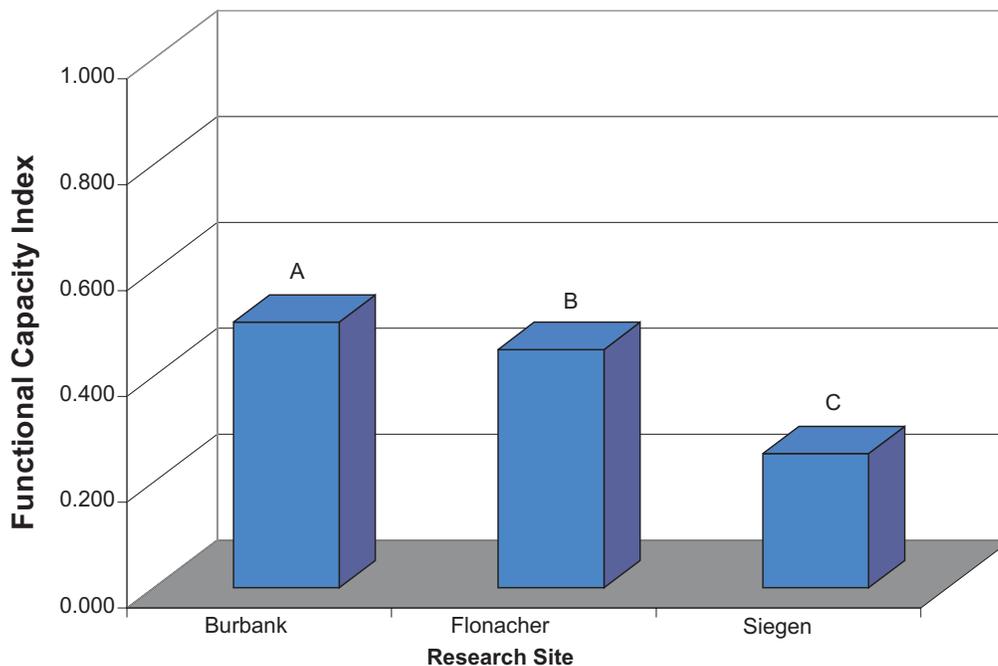


Figure 6. Mean Wildlife Habitat Provision FCIs of Urban Forested Wetlands of East Baton Rouge Parish, Louisiana (means with the same letters are not significantly different from each other at $p = 0.05$).

suburban development affected the spatial scale variables of the wildlife habitat in EBRP. Similar habitat degradation trends by urban development as a threat to wildlife habitat, and native flora and fauna is reported by Marzluff [44].

From the management perspective, the provision of habitat to wildlife function in an urban environment is very important. Urban and suburban fragmented forest patches provide important staging, feeding and resting areas for migratory birds, besides serving as a habitat hotspot for native flora and fauna in an altered landscape [45]. Though the urban forested wetlands function at a lower level in providing habitat for wildlife compared to reference wetlands, these habitat patches needs to be protected/mitigated from further functional losses.

Applicability of the Adjusted Models

The Western Kentucky guidebook served as the basic assessment template for assessing the biotic functions of the urban- forested wetlands of EBRP. Out of the 17 variables given in the Western Kentucky guidebook for the selected functions, standard measurement techniques of 15 variables were found to be applicable to the urban forested wetlands of EBRP. COE used 10 variables associated with the development of wetland vegetation out of the 17 variables of the Western Kentucky guidebook for monitoring the restoration of bottomland hardwood forests in western Tennessee, western Kentucky and eastern Arkansas. The variables were found to be applicable to the low gradient riverine wetlands of the selected sites, although majority of the sites were outside of the reference domain of the Western Kentucky guidebook [46]. The study indicated that the Western Kentucky guidebook's recommended variables associated with the development of biotic characteristics were consistent and applicable to low gradient riverine wetlands in the southeast region [46]. It is not

surprising then that the current study found these same variables applicable to the research sites.

Functional capacity indices for the three functions (Figs. 4 to 6) were determined through the adoption of the adjusted models. The FCIs were significantly different for the three sites based on their existing topo-hydrologic, edaphic and biotic characteristics. The differences between the level of functioning for the three functions were indicative of the difference between the biotic and abiotic characteristics, disturbance level and size of the three sites. On the whole, the functional level of Siegen site was lower than the Burbank and Flonacher sites. This is consistent with the young homogeneous plant community dominated by *Salix nigra*, an indicator of disturbed and newly restoring sites. Siegen site was more disturbed hydrologically, had a non-stratified monotype young forest stand, fragmented and had litter layer/O-horizon mass which significantly reduced its nutrients cycling, maintenance of characteristic plant community and provision of habitat to wildlife functions ($p < 0.01$) compared to Burbank and Flonacher sites.

In conclusion, the average FCIs determined for the selected functions were representative of the sites conditions and accurately captured site specific differences in hydrologic regime, vegetation type, composition, density and stand stratification, litter layer/O-horizon biomass and landscape scale variables. This indicates the suitability and feasibility of the adjusted assessment models for functional assessment of urban forested wetlands in EBRP. Further research on the refinement and validity of these models for urban forested wetlands in the Southeastern US and similar riverine forests elsewhere in the world is recommended.

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ESTIMATION OF SELECTED RESIDUAL ANTIBIOTICS IN MUSCLE, KIDNEY, LIVER, AND EGG OF LAYER CHICKEN

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Received July 2005, accepted October 2005

Communicated by Prof. Dr. A. R. Shakoori

Abstract: The present studies were conducted for the estimation of quinolone residues in our local poultry products. The poultry products included muscle, kidney, liver and egg (yolk and white). The quinolones included in this study were, oxolinic acid, flumequine, enrofloxacin, and ciprofloxacin. An assessment of the variation of each analyte (quinolones antibiotic residues) in these products was made. A comparison was made among the analyte (quinolones) concentrations in different tissues/organs and their internationally permissible safer maximum residue limits (MRLS'). Infra Red spectra were used to detect the presence and identification of different quinolones. HPLC with ODS Column and U.V. detector was used for the quantification.

Keywords: Antibiotic residues, Infra Red spectroscopy, U.V. spectroscopy and High Pressure Liquid Chromatography

Introduction

Pakistan is one of the developing states of the third world. Similarly its frontiers of industry, trade and economy are in a developing state. One of these industries is the poultry industry which, though not advanced, yet has an investment of Rs.65 billion that will come up to Rs.71 by the next year. We have 15000/- poultry farms, producing 370000/- metric tonnes poultry meat and 48.60 million poultry eggs. Seventy percent of the total antibiotics produced today are fed to poultry birds and livestock. U.S senate has passed a bill No. S-2508 for the preservation of antibiotics for human treatment through an act of 2002, with the risk from pathogenic resistance against the antibiotics. FDA is looking to ban two poultry antibiotics that are members of the family of drugs also used in humans. These are sarafloxain and Enrofloxacin (ENRF). The concern is mainly due to the rise of fluoro-

quinolone resistant pathogens called campylobacter bacteria. Pathogens that are transferred to humans when they eat undercooked poultry [1].

Quinolones are applied to prevent infectious diseases. When the lung, urinary or digestive system of an animal is infected, it can be treated by quinolone anti-bacterials by inhibition of DNA gyrase [2,3] which is a type II topoisomerase, essential enzyme for forming DNA supercoils [4].

The quinolones have been classified according to their antibacterial spectrum; potency and pharmacology. There is no widely accepted classification of these at present [4,5]. These are divided into two categories; the first generation quinolones include, nalidixic acid (NAL acid), oxolinic acid (OXOLN acid), Flumequine (FLUM) and piromidic acid (PIRM acid), which have good antibacterial activity against gram-

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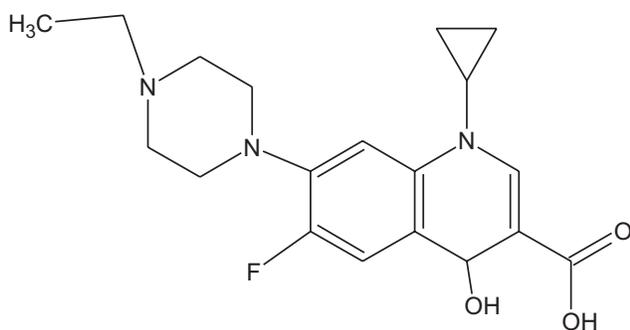
negative bacteria [6]. Their antibacterial effect is no longer good as drug resistant bacteria have evolved [7]. The second category; (the second and third generation) includes fluoroquinolones containing fluorine at C-6 position and piperazinyl at C-7 position, such as marbofloxacin (MARBF), ofloxacin (OFLX), danofloxacin (DNF), ENRF, CPRF, sarafloxacin (SRF), norfloxacin (NORF), and levofloxacin (LEVOF). These have broad antibacterial spectrum. They are safe drugs. These are effective against gram positive, gram-negative bacteria and mycoplasma. So their antibacterial activity is better [2,7,8].

In October 2000, FDA proposed banning the use of fluoroquinolones in poultry claiming that these cause infections by resistant bacteria [9]. Studies have demonstrated unmistakable links between the use of antibiotics as sub-therapeutic

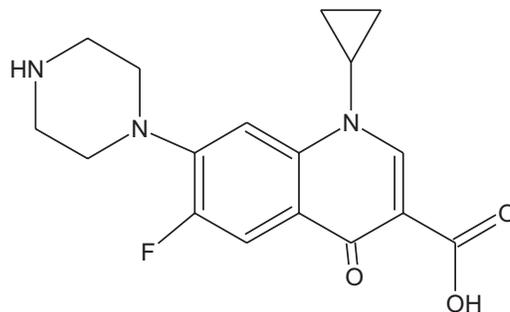
growth promoters and the prevalence of resistant bacteria against fluoroquinolones, which are used in poultry feed [10].

Different international fora have set up maximum permissible and safer antibiotic residue limits [11,12,13]. Hard water carries calcium and magnesium which affect feed efficiency and well being of layers. The varying underground water table changes the contents of metals and non-metal impurities.

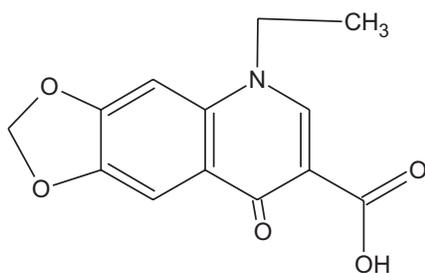
The effect of cooking on the decomposition and concentration of FLUM and OXLN acid was observed. It showed that cooking temperature had no effect but concentration of these quinolones increased by diffusion from the kidney and liver [14]. Flumequine is used to treat poultry cholera but it does not dissolve in water sufficiently at



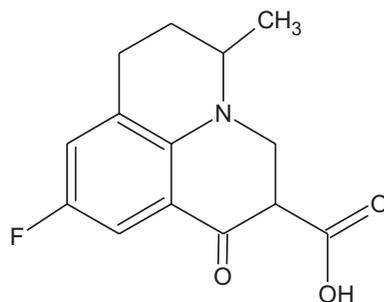
*Enrofloxacin



*Ciprofloxacin



Oxolinic Acid



*Flumequine

Fig. 1. The Chemical structure of quinolone (*=fluoroquinolones).

the stomach and intestinal pH. But it dissolves in water at pH 10. To dissolve it under digestive and assimilative condition of the food, Kitasamycin is added which reacts with it [15]. Minimum inhibitory concentrations (MIC) of 21 antimicrobial agents including FLUM, NAL, OXOLN, Oxytertracycline (OTC), ENRF were estimated against the avian mycoplasma, in Taiwan. All of the field isolates were highly sensitive with (MIC 50µg/ml) [16]. OXOLN, FLUM and OTC reduced the transformation of fatty acids in marine fish farm sediment from a simulated abandoned site. These change humus into natural less fertile soil [17]. The residual amounts of antibiotics in poultry habitualize the microorganisms against themselves. This potential effect is transferred to the poultry product users in the environment and the ecological system contaminated first with the residues and then with the dangerously resistant pathogens. Moreover in the tropical and sub-tropical type of environment such as in Pakistan, high temperature is unable to disintegrate the fluoroquinolones and quinolones, even cooking cannot modify them, hence the increasing no of resistant pathogens is a growing danger to humans as well as to the other animal health. So, it stifles the economy and trade if breakouts of the diseases occur.

Materials and Methods

The samples were collected randomly from local markets situated in Lahore. The layer birds and their eggs were obtained from local markets in Lahore. The samples were collected during the summer of 2004.

HPLC systems consisted of LC- 9A pump (Shimadzu), SPO-6AB, UV-visible spectrophotometric detector (Shimadzu), CTO 6A oven (Shimadzu), SCL -6B system controller, and CSW-32 software. The ultrasonic cleaner (EYELA Tokyo Rikakai Co-Ltd) was used for

degassing the samples, standard and mobile phase. Sartorius filtration assembly (Germany) was used for filtration with nylon filter of 0.45µm pore size (Germany). PH of the mobile phase was set by pH meter (Hanna HI-8418). The extractants, solvents and mobile phase constituents were of HPLC grade.

Extraction of quinolones from layer meat and eggs

Samples of egg yolk and white, liver and muscle (10g of each) were used extraction of quinolones. Phosphate buffer with acetonitrile was added to each sample and magnetic stirring was performed for extraction. The samples were filtered by Whatman filter paper. This procedure was repeated thrice for each sample. Then activated charcoal was used for decoloration and anhydrous sodium sulphate was used for dehydration of the sample. The defatting was done by n-hexane saturated with acetonitrile in a separation flask. Each sample was made up to 50ml by addition of de-ionized water and cleaning was performed with cation and anion exchange bond elute cartridges. The cleaned samples were dried on a water bath and reconstituted with each mobile phase up-to 2 ml followed by high pressure liquid chromatographic analysis.

Extraction of fluoroquinolones from egg

Egg yolk and white (10g) was weighed and homogenized with 2% acetic acid in CH₃CN. Anhydrous sodium sulphate was used for dehydration of the samples before centrifugation. The supernatant was filtered through anhydrous sodium sulphate and washed with acetonitrile. Acidic quinolones were extracted in the same manner as above but without the addition of acetic acid. Acetonitrile was evaporated to dryness and redissolved in 0.05 M disodium hydrogen phosphate (pH 4).

Cleaning of samples

A Bond Elute strong cation exchange (SCX 500mg, Varian) solid phase extraction (SPE) cartridge was conditioned with acetonitrile-glacial acetic acid (95+5 v/v). More acetic acid (5ml) was added to the extract which was then applied to the cartridge. The cartridge was washed sequentially with acetone (2.5ml), (methanol 5ml) and (acetonitrile 5ml). The cartridge was eluted with methanol-35% ammonia solution (95+5 v/v, 5ml) into a test tube. The eluate was dried and reconstituted with HPLC mobile phase. Acid quinolones were cleaned with AGMP-1 resin (Bio-Rad, Richmond (CA, USA) by slurring it in water and packed into empty column and poultry sample extracts were applied to the cartridge. The cartridge was washed with water, methanol, and acetonitrile 5ml each, sequentially it was then eluted with 2x5 ml methanol-acetic acid (95+5 v/v). The eluate was dried and redissolved in mobile phase [18].

HPLC determination

HPLC mobile phase for ENRF and quinolone acids consisted of acetonitrile phosphate buffer with 3.5mM SDS (35:65) at pH 3. The mobile phase for CPRF consisted of CH₃CN: phosphoric acid: deionised water (200:2:800). The ODS column was used. The injection volume was 20 μ l. The UV detector at ambient temperature was used. The flow rates and detection wavelengths for CPRF and ENRF were 1.5ml and 278 nm and 1ml and 284 nm, respectively. The quinolone carboxylic acid OXOLN acid and FLUM were determined with the same apparatus as that for basic quinolones but the mobile phase consisted of 0.01 M oxalic acid: acetonitrile: methanol (6:3:1). The wavelengths of detection were 241 nm and 267 nm for FLUM and OXOLN acid, respectively.

Identification and Quantification

Quinolones (CPRF, ENRF, OXOLN acid and FLUM) were identified in the sample by

Table 1.
Maximum Residue Limits (MRL's) for quinolones in force in Europe [11,12,13].

Substance	Marker Residue	Species	MRL's (μ g/kg)	Tissue
Enrofloxacin	Sum of enrofloxacin & ciprofloxacin	Bovine	30	Muscle
		Porcine	30	Liver
		Poultry	30	Kidney
Ciprofloxacin	Sum of enrofloxacin & ciprofloxacin	Bovine	30	Muscle
		Porcine	30	Liver
		Poultry	30	Kidney
Flumequine	Flumequine	Bovine	50	Muscle
		Ovine	100	Liver
		Porcine	300	Kidney
		Poultry	50	Fat, Skin
		Salamander	150	Muscle, Skin

comparing IR spectra, and retention times of standards with those of unknown substances in the samples using their respective mobile phases in the case of HPLC with U.V. detection. Estimations were made (1) by using standard calibration curves, and (2) by using the relation of peak area per ppm calculations from the standard calibration curve values of peak area/ppm calculation from the standard calibration and concentrations.

Statistical Analysis

The statistical parameters of range, range size and confidence level are all important in this analysis. The variation of the residual amounts of quinolones in different samples of the bird's organ/tissue differ very widely. These have to be described precisely and comprehensively. Hence the mean residue concentration of quinolones is described at 95% confidence level. For example layer liver concentration of ENRF was 1357.15 ± 342.32 at this confidence level. It means that ENRF residues were found in the narrower range deviating positively and negatively by $342.32 \mu\text{g}$ from the mean value $1357.15 \mu\text{g} / \text{Kg}$ with 95% probability. The ranges described in the Tables are significant because these give information of minimum and maximum limits of residues found actually in the samples. The range sizes of different quinolones residues give a patch of concentration irrespective of their upper and lower limits. It also is an index of the bulk of variance. The range is related to standard deviation by the following relation:

$$S_r = R K_R$$

S_r , R and K_R are standard deviation of the population, range and range deviation factor respectively. As the number of samples decreases, the efficiency of the range decreases relative to the standard deviation. These parameters are also an index of the depository and excretory

behaviour of the residues of quinolones in the poultry birds. The confidence limits could be calculated using S_r obtained from the range. The confidence limit, mean concentration and range are interrelated as follows.

$$\text{Confidence limit} = x \pm R t_r$$

Where x , R and t_r are mean concentration, range and range confidence factor respectively. As all these statistical values are interrelated, these affect one another. To describe the analytical data having some degree of variance like that of quinolone residues, with full precision, we have to use the above parameters [19].

Results and Discussion

Tables 2-7 show that ENRF occurs most abundantly and widely in the poultry products. The order of abundance of the quinolones in decreasing order as found tissue/organ wise was: ENRF > CPRF > FLUM > OXOLN acid.

The concentration of the three residues according to tissue/organ was ENRF: Layer Kidney > Layer Liver > Layer "egg", > Layer Muscle and CPRF: Layer Liver > Layer Kidney > Layer Muscle > Layer "Egg" and FLUM: Layer Kidney > Layer Liver. FLUM was absent in layer muscle & "egg". OXOLN Acid was not detected in any of the samples.

The higher values of RSD (relative standard deviation) for kidney and "egg" (43.784 and 56.852%) in case of ENRF show widely varying concentrations in kidneys and "eggs" of the layers (Table 2).

The highest value (13.47%) of RSD for kidney in case of CPRF shows that CPRF (Table 3) concentration differs most widely in kidney than in liver muscle and "egg". The FLUM

Table 2.
Various parameters of enrofloxacin in layer tissues/organs and “egg”.

Sample	Range Size R (µg/kg)	Standard Deviation (S)	Coefficient of variation	Mean residue conc. (µg/kg) at 95% Confidence level
Layer Liver	742.93	342.00	25.223	1357.15 ± 342.32
Layer Kidney	2932.70	1224.002	43.784	2795.57 ± 1224.00
Layer Muscle	576.71	206.997	29.618	698.90 ± 206.99
Layer “Egg”	1279.476	496.237	56.852	872.86 ± 496.24

Table 3.
Various parameters of ciprofloxacin in layer tissues/organs and “egg”.

Sample	Range Size R (µg/kg)	Standard Deviation (S)	Coefficient of variation	Mean residue conc. (µg/kg) at 95% Confidence level
Layer Liver	72.06	28.67	12.27	233.57 ± 28.669
Layer Kidney	48.31	18.95	13.47	140.67 ± 18.948
Layer Muscle	16.62	6.644	7.59	87.43 ± 6.644
Layer “Egg”	9.98	4.214	5.93	71.06 ± 4.214

Table 4.
Various parameters of flumequine in layer tissues/organs and “egg”.

Sample	Range Size R (µg/kg)	Standard Deviation (S)	Coefficient of variation	Mean residue conc. (µg/kg) at 95% Confidence level
Layer Liver	70.00	29.07	126.33%	23 ± 29.07
Layer Kidney	100.08	41.55	117.94%	35.23 ± 41.55
Layer Muscle	Nil	Nil	Nil	Nil
Layer “Egg”	Nil	Nil	Nil	Nil

Table 5.
Enrofloxacin in layer tissues/organs and “egg”.

Parameters	Layer Liver	Layer Kidney	Layer Muscle	Layer “Egg”
Mean Residue level (µg/kg)	1357.15	2795.57	698.898	872.86
No of Obs. (n)	5	5	5	5
Deviating % age from MRL	100	100	100	100
Range (µg/kg)	974.23-1717.16	1629.41-4562.11	425.00-1001.71	393.426-1672.902

Table 6.
Ciprofloxacin in layer tissues/organs and “egg”.

Parameters	Layer Liver	Layer Kidney	Layer Muscle	Layer “Egg”
Mean Residue level (µg/kg)	233.568	140.666	87.434	71.066
No of Obs. (n)	5	5	5	5
Deviating % age from MRL	100	100	100	100
Range Size (µg/kg)	190.08-262.14	124.23-172.54	80.18-96.80	66.05-76.03

Table 7.
Flumequine in layer tissues/organs and “egg”.

Parameters	Layer Liver	Layer Kidney	Layer Muscle	Layer “Egg”
Mean Residue level (µg/kg)	23.002	35.226	Nil	Nil
No of Obs. (n)	5	5	5	5
Deviating% age from MRL	0.00	0.00	0.00	0.00
Range Size (µg/kg)	0.0-70	0.0-100.08	Nil	Nil

occurs almost equally widely (RSD's 126.33% and 117.94%) in liver and kidney, respectively with smaller mean concentration values of 23.002 and 35.23 µg/kg for liver and kidney respectively (Table 4). ENRF and CPRF show 100% deviation from the internationally accepted MRL's (Maximum Residue Limits) (Table 5-6). Hence, these require longer washing out periods and dose sophistication for poultry chicken during summer. FLUM residues occur only in liver and kidney but only in 60% samples and to the extent that is less than MRL's. (Table 7). OXOLN acid residues were not detected in any layer sample. Range sizes of the residual antibiotics depend mostly on the water intake, nature of water, its metallic content and pH of the poultry water and feed. The least concentrations of FLUM residues show that working pH of kidney and urine is most favorable for the acidic FLUM secretion. On the other hand, the larger values of range, range sizes and residual concentrations for basic fluoroquinolones; CPRF and ENRF are due to their (1) heavy doses, and (2) decreased renal secretions due to their larger lipophilicities [20]. Large range sizes and concentration of kidney residues show that basic quinolones are not ionized properly due to unfavourable renal pH and for elimination from the kidneys these have to be in ionized form. The larger the difference in residual range sizes between the liver and the kidney, the greater the decrease in their elimination. Owing to their smaller residual amounts in the kidney compared to the liver, the CPRF show increased secretion in contrast to ENRF whose concentration in the kidney exceeds that in the liver. This is also due to more lipophilicity of ENRF than that of CPRF. To decrease kidney, liver and muscle residues, the washing out time periods should be increased along with the intake of Vitamin C which will render the kidney to work in still more acidic medium and increase the secretion of ENRF and CPRF [21]. The absence of FLUM in muscle and

“egg” shows an ideal condition which should also be achieved in case of CPRF and ENRF. The pH, the heavy metals and washing out times should be monitored rigorously. Dose sophistication and nature of the drug should also be accounted for.

Out of the detected analytes, ENRF and CPRF residues show excessive concentration levels (Tables 2,3). These must be monitored in the poultry industry. Washing out period must be enhanced to satisfactory limits. Dose sophistication must be ensured with respect to seasonal variation. Vitamin C, feed and secretion pH must be optimized. FLUM and OXOLN acid residues obey international regulations. But if the monitoring of the ENRF and CPRF concentration levels are neglected in our poultry industry, it is likely to undergo resistance against *Pseudomonas*, *Staphylococcus aureus*, *P. aeruginosa*, *Enterobacteriaceae* and *Salmonella*.

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LORENTZ TRANSFORMATIONS ARE UNABLE TO DESCRIBE THE RELATIVISTIC DOPPLER EFFECT

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Received January 2006, accepted February 2006

Communicated by Dr. M. Iqbal Choudhary

Abstract: We know paradoxes do not exist in nature and a complete theory does not include them. We only need to use our intuition to see that there are no logical inconsistencies. In this context, the relationship between the Relativistic Doppler Effect (RDE) and Lorentz Transformations (LT) exhibits logical inconsistencies. It means that LT misrepresents reality and describes no physical effects. In this paper we will explain how to eliminate such logical inconsistencies (contradictions).

Keywords: Doppler effect, speed of light, time dilation, special relativity theory

Introduction

In his well known article on “Special Relativity” Einstein succeeded in deriving LT [1], and then deriving the relativistic Doppler relations based on these transformations. Therefore relativistic Doppler effect (RDE) is related to the time dilation effect [2]. SRT accounts for various kinematical effects, like length contraction and time dilation. Several questions arise when examining this kinematical effect and many contradictions exist. Moreover SRT and relativistic Doppler relations are incompatible. In this regard we will see that the asymmetry of kinematical time dilation effect derived by LT makes it difficult to reconcile LT effects and RDE completely.

In this paper, we depend on the method in [3,4], in which it is shown that the Doppler calculation procedure as well as interpretation is possible only with the help of the Lorentz force law and the relativity principle. This will end the role of the Lorentz transformation (LT) and of time dilation in RDE.

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Einstein’s Method in Deriving Doppler’s Formula

It is well known that the color of light rays coming out of a moving source towards the observer tend to be blue shifted (i.e. high frequency), whereas a ray exiting a moving source in the opposite direction tends to be red shifted (i.e. low frequency). The diversity of possibilities along with the existence of ether between the source and the observer leads to four possibilities, explained as follows.

1. If the source is receding/approaching from the rest observer, the frequency that observer sees is classically

$$f' = \frac{f_0}{1+(u/c)}, \quad f' = \frac{f_0}{1-(u/c)} \quad (1a,1b)$$

2. If the observer is receding/approaching from the rest source, the frequency that observer sees is classically

$$f' = f_0 (1 - \frac{u}{c}), \quad f' = f_0 (1 + \frac{u}{c}) \quad (2a,2b)$$

By excluding the idea of ether, Einstein has reduced these four possibilities to only two, namely,

$$f' = \sqrt{1 - \frac{u^2}{c^2}} \frac{f_0}{1 + \frac{u}{c}} = f_0 \sqrt{\frac{1 - u/c}{1 + u/c}} \quad (3a)$$

$$f' = \sqrt{1 - \frac{u^2}{c^2}} \frac{f_0}{1 - \frac{u}{c}} = f_0 \sqrt{\frac{1 + u/c}{1 - u/c}} \quad (3b)$$

Relativity principle makes it easier to use when it considers these two possibilities as actually one single possibility, and we get the second possibility through converting the speed sign in the first.

We can see the difference between the classical Doppler effect applied to light waves and RDE. It makes no sense to talk about the velocity of either the source or the observer relative to the medium as one does in ether. One considers only the relative velocity u between the source and the observer as the RDE includes time dilation, i.e. the RDE includes also the transverse Doppler effect (TDE).

Einstein obtained in his work [1] the following formula:

$$f' = f_0 \frac{\sqrt{1 - (u^2/c^2)}}{1 + (u/c) \cos \theta} \quad (4)$$

where u is the speed of source, θ is the direction of travel.

There are two particular cases that lead to simplifications. The first is motion along the line of sight-the longitudinal relativistic Doppler effect, where

$$1- \quad f' = \sqrt{1 - \frac{u^2}{c^2}} \frac{f_0}{1 + \frac{u}{c}} = f_0 \sqrt{\frac{1 - u/c}{1 + u/c}} \quad (5a)$$

Had the source been approaching from the observer, then

$$2- \quad f' = \sqrt{1 - \frac{u^2}{c^2}} \frac{f_0}{1 - \frac{u}{c}} = f_0 \sqrt{\frac{1 + u/c}{1 - u/c}} \quad (5b)$$

The other special case is that of transverse motion across the line of sight. In this case

$$3- \quad f' = f_0 \sqrt{1 - \frac{u^2}{c^2}} = \frac{f_0}{\gamma} \quad (5c)$$

Eqs. (5a, 5b) have classical analogues in Eqs. (1a, 1b).

The frequency is red shifted due to the dilation of the source time, Eq. (5c), and this effect (TDE) corresponds to the time slowing down on the source moving clock.

In SRT's formalism the key effect for RDE is time dilation, which plays an important part in modern physics. Therefore Eq. (5c) is considered a unique feature of SRT and is related to the dilation of time only for the moving source. Therefore a statement was raised in textbooks that "The TDE should not occur in classical physics". This statement is not correct in some models [5,6], and it is not correct if one derives TDE without ether theory or any relativistic assumption such as time dilation as we will do in the present paper.

Einstein believed that the general formula Eq. (4) which he deduced is an appropriate

formula for the two cases, the source is moving and observer is at rest, or the source is at rest and the observer is moving. As remarked in Einstein's method, RDE and TRD modes treat only source receding/approaching from the rest observer. However, an accurate analysis of Eq. (4) by using LT would reveal that there is an important contradiction between RDE and LT.

According to the relativity principle, Eq. (4) could be written for the case of observer in motion as,

$$f' = f_0 \gamma \left(1 - \frac{u}{c} \cos \theta\right)$$

If the motion is normal to the line connecting source and observer, we then obtain from the last equation

$$f' = \gamma f_0$$

This equation shows a time contraction, instead of a time dilation as in Eq. (5c). We know that time contraction does not exist in SRT but the symmetry effect of TDR requires a time contraction.

We will see now that the asymmetry of kinematical time dilation effect derived by LT makes it difficult to reconcile LT effects and RDE completely.

Derivation of RDE and TDE from Lorentz Transformations

Assume two inertial frames S and S' , a source with frequency f_0 in the moving frame, an observer in the rest frame, and the source approaching with the relative velocity $u \parallel ox$ from the observer.

The position of the radiation frequency of moving source is described by

$$x = ct, \quad x' = ct' \quad (6)$$

where (x, t) and (x', t') are spatial and time intervals. Then, we apply LT

where (x, t) and (x', t') are spatial and time intervals. Then, we apply LT

$$x' = \gamma (x - ut), \quad t' = \gamma \left(t - \frac{u}{c^2} x\right) \quad (7)$$

we have

$$t' = \gamma t \left(1 - \frac{u}{c}\right) \quad (8)$$

The frequency is derived as the inverse of time, i.e.

$$t = \frac{1}{f_0}, \quad t' = \frac{1}{f'} \quad (9)$$

If we insert Eq. (9) in (8), we find

$$\begin{aligned} f' &= \frac{f_0 \sqrt{1 - (u^2/c^2)}}{1 - (u/c)} \\ &= f_0 \sqrt{\frac{1 + (u/c)}{1 - (u/c)}} \end{aligned} \quad (10)$$

Had the source been receding from the observer, then by replacing u with $-u$ in Eq. (10), we have

$$f' = f_0 \sqrt{\frac{1 - (u/c)}{1 + (u/c)}} \quad (11)$$

Let the reference time in S be t_0 . The reference time in S' is defined by Eq. (7), i.e.,

$$t' = \gamma t_0 \quad (12)$$

Using (9) in (12), we get

$$f' = \frac{f_0}{\gamma} = f_0 \sqrt{1 - (u^2/c^2)} \quad (13)$$

Eqs. (10,11 and 13) are the relativistic Doppler shift derived by Einstein [1], but now is derived from LT directly. According to relativity principle, we can also consider the frame to be co-moving with the source and receding/approaching the observer. Then Eq. (7) could be written as

$$x = \gamma(x' + ut') , \quad t = \gamma(t' + \frac{u}{c^2}x') \quad (14)$$

Inserting (6) in (14), we obtain

$$t = \gamma t' (1 + \frac{u}{c})$$

Then using (9) in the last equation, we have

$$f_0 = \frac{f' \sqrt{1 - (u^2/c^2)}}{(1 + \frac{u}{c})}$$

or

$$\begin{aligned} f' &= \frac{f_0 (1 + \frac{u}{c})}{\sqrt{1 - (u^2/c^2)}} \\ &= f_0 \sqrt{\frac{1 + u/c}{1 - u/c}} \end{aligned} \quad (15)$$

Had the observer been receding from the source, then by replacing u with $-u$ in Eq. (15), we have

$$f' = f_0 \sqrt{\frac{1 - (u/c)}{1 + (u/c)}} \quad (16)$$

If the motion is normal to the line connecting source and observer, we then obtain

$$f' = \frac{f_0}{\sqrt{1 - \frac{u_r^2}{c^2}}} = \gamma f_0 \quad (17)$$

where in normal movement, the radial component is zero, $u_r = 0$, and since

$$u^2 = u_t^2 + u_r^2 = u_t^2$$

we obtain also a change in frequency as per Eqs. (17). So, the Doppler effect exists even though there is no component of relative motion along the line of sight. The reference time of the moving observer becomes long, i.e. according to Eqs. (9) and (17), we have

$$t' = t_0 / \gamma \quad (18)$$

The reference time of the moving observer decreases (time contraction). Thus the frequency of the light source that is seen by the moving observer increases as in Eq. (15) and decreases as in Eq. (16).

Eqs. (15,16 and 17) were not derived by Einstein since the case of rest source and moving observer require time contraction as in Eq.(18) while the kinematical effect derived by LT is time dilation and not time contraction. Thus, the Lorentz transformation (7) and (14) have been used for the calculation of the so called relativistic effect, and we now know that they give the possibility to calculate the relativistic effect only if LT has time contraction as in Eq. (18). This means that LT misrepresents reality and reflects no physical effects [3,4].

Derivation of RDE and TDE from Lorentz Force

Now assume that a particle q in frame S as ion (source) emits a light wave (photon) that

moves in a direction that makes an angle θ with the positive ox axis. The light is received at the observer in frames S' at an angle θ' relative to the ox' axis. In [3,4] we have derived the following relation:

$$v' = \gamma \left(1 - \frac{u}{c} \cos \theta\right) \quad (19a)$$

But the connection between the two frequencies in frames S and is S' given also by

$$v = \gamma' \left(1 + \frac{u}{c} \cos \theta'\right) \quad (19b)$$

If we consider the frame S to be co-moving with the source and receding /approaching observer, Eq. (19a) becomes

$$1-\theta = \theta' = 0^\circ \text{ i.e. } v' = v_0 \gamma \left(1 - \frac{u}{c}\right) = v_0 \sqrt{\frac{1 - \frac{u}{c}}{1 + \frac{u}{c}}} \quad (20a)$$

$$2-\theta = \theta' = 180^\circ \text{ i.e. } v' = v_0 \gamma \left(1 + \frac{u}{c}\right) = v_0 \sqrt{\frac{1 + \frac{u}{c}}{1 - \frac{u}{c}}} \quad (20b)$$

Eqs. (20a,20b) do not have relativistic analogues, but have classical analogues in Eqs. (2a,2c). According to relativity principle, we can also consider the frame to be co-moving with the observer and receding /approaching source, then Eq. (19b) could be written as

$$v' = \frac{v_0 \sqrt{1 - \frac{u^2}{c^2}}}{1 + \frac{u}{c} \cos \theta'} \quad (20c)$$

Hence

$$1-\theta = \theta' = 0^\circ \text{ i.e. } v' = \frac{v_0 \sqrt{1 - \frac{u^2}{c^2}}}{1 + \frac{u}{c}} = v_0 \sqrt{\frac{1 - \frac{u}{c}}{1 + \frac{u}{c}}} \quad (21a)$$

$$2-\theta = \theta' = 180^\circ \text{ i.e. } v' = \frac{v_0 \sqrt{1 - \frac{u^2}{c^2}}}{1 - \frac{u}{c}} = v_0 \sqrt{\frac{1 + \frac{u}{c}}{1 - \frac{u}{c}}} \quad (21b)$$

Eqs. (21a, 21b) are identical to Eqs. (5a, 5b) in SRT, and the classical analogues are (1a, 1b).

If the velocity of the observer/source is perpendicular to the line of sight, then we have from Eq. (19a),

$$\theta = \theta' = 90^\circ \text{ i.e. } v' = \frac{v_0}{\sqrt{1 - \frac{u^2}{c^2}}} = \gamma v_0 \quad (22a)$$

and from Eq. (20c), we have

$$\theta = \theta' = 90^\circ \text{ i.e. } v' = v_0 \sqrt{1 - \frac{u^2}{c^2}} = \frac{v_0}{\gamma} \quad (22b)$$

Formula (22a) has been confirmed in a Mossbauer experiment with a moving detector [7], and formula (22b) was early verified by Ives and Stilwell experiments [8,9].

In [3] we have shown, that had it not been for the existence of formula (22a) and (22b) together, there would be no equality between the two formulas (20a) and (21a), and the two formulas (20b) and (21b). This means that formula (22a) exists since this formula is not the outcome of SRT due to time contraction. This

would mean that LT is unable to describe a well known physical reality, namely the Doppler effect.

We turn to Eqs. (19a,20c) i.e.

$$v' = \gamma_0 \left(1 - \frac{u}{c} \cos \theta\right), \text{ and } v' = \frac{v_0 \sqrt{1 - \frac{u^2}{c^2}}}{1 + \frac{u}{c} \cos \theta'} \quad (23)$$

Einstein has used LT to give a new interpretation of the astronomically observed phenomenon of aberration. This was indeed already discovered in the early eighteenth century by Bradley [10]. He gave an explanation on the basis of the ether theory and the finite velocity of light. Hence, in this paper the explanation did not have to wait for the construction of LT. The two angles in Eq. (23) differ when we include the effect of aberration. If we let θ denote the angle with respect to the source's frame and θ' denotes the angle with respect to the observer's frame, then we have

$$\frac{v_0 \sqrt{1 - \frac{u^2}{c^2}}}{1 + \frac{u}{c} \cos \theta'} = \gamma_0 \left(1 - \frac{u}{c} \cos \theta\right)$$

or

$$1 + \frac{u}{c} \cos \theta' = \frac{1 - \frac{u^2}{c^2}}{1 - \frac{u}{c} \cos \theta} \quad \text{i.e.}$$

$$\cos \theta' = \frac{\cos \theta - \frac{u}{c}}{1 - \frac{u}{c} \cos \theta} \quad (24)$$

Eq. (24) describes the aberration of light. Star aberration arises because the observer moves with the orbital speed of the Earth. If, as SRT asserts, the movement of the light source is equivalent to the movement of the observer, star aberration has to arise as in the case when the source moves. However, the observations of binary stars prove that there is no aberration when the stars move.

In conclusion, due to the Eqs. (22a) and (22b) together, the RDE formula for a moving observer can also be written in the form used for a moving source. Certainly, the formula (22a), which does not have an equivalent in the SRT, is very significant for the formula (22b) for the equality of the longitudinal relativistic Doppler effect. That means the longitudinal relativistic Doppler effect for a moving observer can be also written in the form used for a moving source. The kinematical effect derived by LT is time dilation but not time contraction. Thus, the asymmetry of the kinematical time dilation effect derived by LT makes it difficult to reconcile LT effects and RDE completely. These contradictions cannot be removed without excluding LT and deriving RDE and TRD formulas without LT [3,4]. The inherent asymmetry of kinematical time dilation effect were found also recently by V. Sokolov and G. Sokolov [10,11]. Star aberration and the TDR arise as the result of using the Lorentz force and relativity principle. Both effects do not prove that time dilation takes place in moving systems. Both these phenomena contradict SRT and prove it is false.

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LEVERRIER-FADDEEV'S ALGORITHM APPLIED TO SPACETIMES OF CLASS ONE

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Received October 2005, accepted January 2006

Communicated by Prof. Dr. M. Iqbal Choudhary

Abstract: We explain that the Leverrier-Takeno's method to construct the characteristic polynomial of an arbitrary matrix \tilde{A} , plus the Cayley-Hamilton theorem, it is equivalent to the Faddeev's process to obtain \tilde{A}^{-1} . We apply this algorithm to the second fundamental form of a spacetime embedded into flat 5-space.

Keywords: Cayley-Hamilton theorem, Faddeev algorithm, Leverrier-Takeno method, Riemannian 4-spaces of class one.

Introduction

For any matrix $\tilde{A}_{n \times n} = (A^i_j)$ its characteristic equation:

$$\lambda^n + a_1 \lambda^{n-1} + \dots + a_{n-1} \lambda + a_n = 0 \quad (1)$$

can be obtained, through several procedures [1-5], directly from the condition $\det(A^i_j - \lambda \delta^i_j) = 0$. The approach of Leverrier-Takeno [1,6-9] is a simple and interesting technique to construct (1) based in the traces of the powers \tilde{A}^r , $r=1, \dots, n$.

On the other hand, it is well known that an arbitrary matrix \mathbf{A} satisfies its characteristic polynomial:

$$\tilde{A}^n + a_1 \tilde{A}^{n-1} + \dots + a_{n-1} \tilde{A} + a_n I = 0, \quad (2)$$

which is the Cayley-Hamilton identity. If \tilde{A} is non-singular (that is, $\det \tilde{A} \neq 0$), then from (2) we obtain its inverse matrix:

$$\tilde{A}^{-1} = -\frac{1}{a_n} (\tilde{A}^{n-1} + a_1 \tilde{A}^{n-2} + \dots + a_{n-1} I), \quad (3)$$

where $a_n \neq 0$ because $a_n = (-1)^n \det \tilde{A}$.

Faddeev [10-13] proposed an algorithm to determine \tilde{A}^{-1} in terms of \tilde{A} and their traces. Here we exhibit that (3) coincides with the Faddeev's result if we employ the Leverrier-Takeno's formulae for a_j . After, we apply this analysis to second fundamental form \mathbf{b} governing the extrinsic geometry of Riemannian 4-spaces of class one (that is, 4-spaces embedded into (E_5) [14-18]. The corresponding eq. (3) leads to an original expression for its inverse matrix as function of \mathbf{b} and the double dual of Riemann tensor (projection of it onto the Levi-Civita tensor) [18,19]. Normally the Leverrier-Takeno-Faddeev technique is considered useful only in problems of numerical analysis, but the aim of our work is to show the importance of this algorithm in geometrical theories as general relativity, with potential applications to several physical fields.

Leverrier-Takeno and Faddeev methods

If we define the quantities

$$a_0 = 1, \quad s_r = \text{tr } \tilde{A}^r, \quad r = 1, 2, \dots, n \quad (4)$$

then the process of Leverrier-Takeno[1,6-9] implies (1) wherein the a_i are determined with the recurrence expression

$$\begin{aligned} r a_r + s_1 a_{r-1} + s_2 a_{r-2} + \dots + s_{r-1} a_1 + s_r &= 0, \\ r &= 1, 2, \dots, n \end{aligned} \quad (5)$$

Therefore

$$\begin{aligned} a_1 &= -s_1, & 2! a_2 &= (s_1)^2 - s_2, \\ 3! a_3 &= -(s_1)^3 + 3s_1 s_2 - 2s_3, \end{aligned} \quad (6)$$

$$4! a_4 = (s_1)^4 - 6(s_1)^2 s_2 + 8s_1 s_3 + 3(s_2)^2 - 6s_4, \text{ etc.}$$

In particular $\det \tilde{A} = (-1)^n a_n$, that is, the determinant of any square matrix only depends on the traces s_r , which means that \tilde{A} and its transpose have the same determinant.

The Faddeev procedure [10-13] to obtain \tilde{A}^{-1} is a sequence of algebraic computations on the powers \tilde{A}^r and their traces. In fact, his algorithm is given by the instructions

$$\begin{aligned} \tilde{A}_1 &= \tilde{A}, \quad q_1 = \text{tr } \tilde{A}_1, \quad \tilde{B}_1 = \tilde{A}_1 - q_1 \tilde{I}, \\ \tilde{A}_2 &= \tilde{B}_1 \tilde{A}, \quad q_2 = \frac{1}{2} \text{tr } \tilde{A}_2, \quad \tilde{B}_2 = \tilde{A}_2 - q_2 \tilde{I}, \\ \tilde{A}_{n-1} &= \tilde{B}_{n-2} \tilde{A}, \quad q_{n-1} = \frac{1}{n-1} \text{tr } \tilde{A}_{n-1}, \quad \tilde{B}_{n-1} \\ &= \tilde{A}_{n-1} \end{aligned} \quad (7)$$

$$\tilde{A}_n = \tilde{B}_{n-1} \tilde{A}, \quad q_n = \frac{1}{n} \text{tr } \tilde{A}_n$$

Then

$$\tilde{A}^{-1} = \frac{1}{q_n} \tilde{B}_{n-1} \quad (8)$$

For example, if we apply (7) for $n = 4$, then it is easy to see that the corresponding q_r imply (6) with $q_j = -a_j$, and besides (8) reproduces (3). By mathematical induction one can prove that (7) and (8) are equivalent to (3), (4) and (5), showing thus that the Faddeev technique has its origin in the Leverrier-Takeno method plus Cayley-Hamilton theorem.

Spacetimes of class one

A R_4 can be embedded into E5 (that is, the 4-space has class one) if and only if there exists the second fundamental form $b_{ca} = b_{ca}$ satisfying the Gauss-Codazzi equations [14-18,20]

$$R_{acij} = \hat{a}(b_{ai} b_{cj} - b_{aj} b_{ci}), \quad (9)$$

$$b_{ij;c} = b_{ic;j} \quad (10)$$

where $\hat{a} = \pm 1$, R_{acij} is the Riemann tensor and ; r means covariant derivative. It is well-known [21] that whenever $\det(b^i_j)$ is different to zero then (9) implies (10). In other words, when a non-singular matrix \mathbf{b} satisfies the Gauss equation, the Codazzi equation is verified automatically. However, in general the construction of \mathbf{b} for a given spacetime should involve the study of both (9) and (10) together.

Employing (9) it is not difficult to deduce the following result [14,15,22].

$$-24 \det(b^i_j) = K_2^* R^{*ijac} R_{ijac} \quad (11)$$

where K_2 is a Lanczos invariant [19,23-25] defined in terms of the double dual of curvature tensor

$${}^* R^{*ij}_{ac} = \frac{1}{4} \eta^{ijrt} R_{rt}{}^{mn} \eta_{mnac}, \quad (12)$$

with η_{jrt} denoting the Levi-Civita symbol, then the Bianchi identities [20] adopt the compact

form [18,19]

$${}^*R^i{}_{jac;i} = 0, \quad (13)$$

The present work deals with the case $K_2 \neq 0$, thus, according to (11) this implies that the inverse matrix $b^{-1}{}_{ij}$ exists. In this situation we employ (2), (6), (9) and (11) to obtain the characteristic polynomial of \mathbf{b} [15,16]

$$\mathbf{b}^4 - b\mathbf{b}^3 - \frac{\varepsilon}{2}R\mathbf{b}^2 - p\mathbf{b} - \frac{K_2}{24}\mathbf{I} = \mathbf{0}, \quad (14)$$

where $R = R^i{}_{ji}$ is the scalar curvature and

$$b = \text{tr } \mathbf{b}, \quad p = \frac{\varepsilon}{3}b^{ac}G_{ac},$$

$$G_{ij} = {}^*R^*{}_{ija} = \text{Einstein tensor}, \quad (15)$$

Then with (3) and (14) we construct the inverse matrix of \mathbf{b}

$$\frac{K_2}{24} b^{-1}{}_{ij} = \varepsilon b_{ir} G^r{}_j - p g_{ij}, \quad (16)$$

In a given spacetime we know the metric tensor g_{ij} , G_{ac} and K_2 ; if besides we have \mathbf{b} and ε , then (15) determines p . Thus (16) gives us \mathbf{b}^{-1} and

$$b^{-1r}{}_r = \text{tr } \mathbf{b}^{-1} = -\frac{24p}{K_2}. \quad (17)$$

On the other hand, the double dual (12) admits the expansion [16,19,23]

$${}^*R^*{}_{jka} = R_{jka} + G_k g_{ja} + G_a g_{jk} - G_a g_{jc} - G_c g_{ja} + \frac{R}{2}(g_c g_{ja} - g_a g_{jc}), \quad (18)$$

Therefore (9), (16), (17) and (18) imply the original relation

$$K_2 b^{-1}{}_{ia} = \delta a^a {}^*R^*{}_{ijac} b^{jc}, \quad (19)$$

which means that \mathbf{b}^{-1} essentially is the projection of \mathbf{b} onto double dual tensor. The Codazzi equation (10) is a differential condition on \mathbf{b} .

However, we searched in the literature some differential restriction on \mathbf{b}^{-1} , but without success in this quest. The importance of (19) is that it, (10) and (13) generate such differential requisite

$$(K_2 b^{-1i}{}_a)_{;i} = 0, \quad (20)$$

which is other original contribution of this work.

The deduction of (19) and (20) shows the usefulness of Leverrier-Faddeev-Takeno expressions in the study of 4-spaces of class one. However, our analysis also is applicable to geometric control theory, quantum information processing, field theory, quantum groups and chaos theory (see [26-32]).

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PARTIAL SUMS OF CERTAIN CLASSES OF MEROMORPHIC FUNCTIONS

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Received January 2006, accepted February 2006

Communicated by Prof. Dr. M. Iqbal Choudhary

Abstract: Using Al-Oboudi differential operator, we study some classes of meromorphic functions. A necessary and sufficient condition for belonging to these classes, coefficients, extremal functions and partial sums are studied.

Keywords: Meromorphic, convex, starlike, convolution, differential operator, extremal points, partial sums, 2000 Mathematics Subject Classification 30C45.

1. Introduction and definitions

Let Σ_p denote the class of functions of the form $f(z) = \sum_{k=p}^{\infty} a_{k+p-1} z^k = \frac{1}{z^p} + \frac{a_0}{z^{p-1}} + \frac{a_1}{z^{p-2}} + \dots, a_{-1} = 1, p \in \mathbb{N} = 1, 2, 3, \dots$ (1.1)

which are regular and multivalent in punctured disk $U = \{z: 0 < |z| < 1\}$.

Then a function f belonging to Σ_p is said to be meromorphic starlike Σ_p^* function if and only if it satisfies

$$\Re \left\{ - \left(\frac{zf'(z)}{f(z)} \right) \right\} > \alpha. \quad (1.2)$$

Also a function f belonging to Σ_p is said to be meromorphic convex Σ_p^* if and only if it satisfies

$$\Re \left\{ - \left(1 + \frac{zf''(z)}{f'(z)} \right) \right\} > \alpha \quad (1.3)$$

Recently, Al-Oboudi [1] defined a differential operator of an analytic function f of the form

$$f(z) = z + \sum_{k=2}^{\infty} a_k z^k \quad (1.4)$$

as follows.

Let

$$D^0 f(z) = f(z) \\ D^1 f(z) = D_\lambda f(z) = (1-\lambda)f(z) + \lambda z f'(z)$$

and

$$D^n f(z) = D_\lambda (D^{n-1} f(z)) = (1-\lambda)D^{n-1} f(z) + \lambda z (D^{n-1} f(z))'$$

for $\lambda \geq 0$ and $n \in \mathbb{N}_0 = \{0, 1, 2, 3, \dots\}$. When $\lambda = 1$, we have Sălăgean differential operator [4]. It can easily be seen that f is given by (1.4). Then

$$D^n f(z) = z + \sum_{k=2}^{\infty} [1 + \lambda(k-1)]^n a_k z^k = f(z) * \left(z + \sum_{k=2}^{\infty} [1 + \lambda(k-1)]^n z^k \right).$$

Applying this differential operator to p -valent meromorphic function in Σ_p , we have

$$D f(z) = \frac{1}{z^p} + \sum_{k=p+1}^{\infty} \left[\frac{1 + (k-1)\lambda}{1 - (p+1)\lambda} \right] a_{k+p-1} z^k$$

and

$$D^n f(z) = \frac{1}{z^p} + \sum_{k=-p+1}^{\infty} \left[\frac{1+(k-1)\lambda}{1-(p+1)\lambda} \right]^n a_{k+p-1} z^k$$

$$= f(z)^* \left(\frac{1}{z^p} + \sum_{k=-p+1}^{\infty} \left[\frac{1+(k-1)\lambda}{1-(p+1)\lambda} \right]^n z^k \right), \quad (1.5)$$

for all and $-p \leq k < \infty$, $p \in N = \{1,2,3,\dots\}$ and $n \in N_0 = \{0,1,2,3,\dots\}$.

Definition 1

A function f of the form

$$f(z) = \frac{1}{z^p} + \sum_{k=-p+1}^{\infty} a_k z^k, \quad (1.6)$$

belongs to $M(n\lambda, \alpha, p)$ if and only if

$$\Re e \left\{ \frac{D^{n+1} f(z)}{D^n f(z)} - (p-1) \right\} < -\alpha \quad (z \in U) \quad (1.7)$$

where and $0 \leq \alpha < p, \lambda \geq \frac{2\alpha(1+p-\alpha)}{p-\alpha} > 0$, and $n \in N_0$.

We can see that for different selections of n, λ and α , we have many different classes of p -valent meromorphic functions.

Definition 2

$$\text{If } f(z) = \frac{1}{z^p} - \sum_{k=-p+1}^{\infty} a_k z^k, a_k \geq 0, \quad (1.8)$$

then $f \in T(n, \lambda, \alpha, p)$ if and only if

$$\Re e \left\{ \frac{D^{n+1} f(z)}{D^n f(z)} - (p+1) \right\} < -\alpha \quad (z \in U)$$

where $0 \leq \alpha < p, \lambda \geq \frac{2\alpha(1+p-\alpha)}{p-\alpha} > 0$, and $n \in N_0$.

2. Inclusion and sufficient conditions

In the next theorem we prove that all functions in the class $M(n\lambda, \alpha, p)$ are p -valent meromorphic starlike of the order α . We need the following lemma.

Lemma 2.1 [2].

Let w be a non constant and regular function in U , $w(0) = 0$. If $|w(z)| \leq 1$ attains its maximum value on the circle $|z| < r < 1$ at z_0 we have, $z_0 w'(z) = kw(z_0)$ where k is real number and $k \geq 1$.

Theorem 2.1

Let $f(z) = \sum_{k=-p}^{\infty} a_k z^k$ be in $M(n\lambda, \alpha, p)$, where $a_{-p} = 1$. Then for all $n \in N_0$ and $0 \leq \alpha < p$ and $\lambda \geq 0$

$$M(n+1, \lambda, \alpha, p) \subseteq M(n, \lambda, \alpha, p).$$

Proof

Let $f \in M(n+1, \lambda, \alpha, p)$. Then

$$\left\{ \frac{D^{n+1} f(z)}{D^n f(z)} - (p-1) \right\} = -\frac{-p-(2\alpha-p)w(z)}{1+w(z)}. \quad (2.1)$$

We have to show that (2.1) implies the inequality

$$\left| \frac{-\frac{D^n f(z)}{D^{n-1} f(z)} + 2(p-1)+1}{\frac{D^n f(z)}{D^{n-1} f(z)} - 1} \right| < 1. \quad (2.2)$$

Let us define w in U by

$$\left\{ \frac{D^n f(z)}{D^{n-1} f(z)} - (p+1) \right\} = -\left[\alpha + (p-\alpha) \frac{1-w(z)}{1+w(z)} \right]. \quad (2.3)$$

$$\left\{ \frac{D^n f(z)}{D^{n-1} f(z)} - (p+1) \right\} = -\frac{1+(2p-2\alpha+1)w(z)}{1+w(z)}. \quad (2.4)$$

Differentiating (2.4) logarithmically, we obtain

$$\left\{ \frac{D^{n+1}f(z)}{D^n f(z)} - (p+1) \right\} = \frac{2\lambda(p-\alpha)zw'(z)}{(1+w(z))(1+(2p-2\alpha+1)w(z))} + \frac{D^n f(z)}{D^{n-1}f(z)} - (p+1)$$

$$= \left\{ \frac{2\lambda(p-\alpha)zw'(z)}{(1+w(z))(1+(2p-2\alpha+1)w(z))} - \alpha - (p-\alpha)\frac{1-w(z)}{1+w(z)} \right\}. \quad (2.5)$$

We claim that $|w(z)| < 1$ in U . For otherwise [by Lemma 2.1] there exists z_0 in U such that

$$z_0 w'(z) = kw'(z_0) \quad (2.6)$$

where $|w(z_0)| = 1$ and $k \geq 1$. From (2.5) and 2.6), we obtain

$$\left\{ \frac{D^{n+1}f(z)}{D^n f(z)} - (p+1) \right\} = -\frac{p+(2\alpha-p)w(z)}{1+w(z)} + \frac{2\lambda(p-\alpha)zw'(z)}{(1+w(z))(1+(2p-2\alpha+1)w(z))} \quad (2.7)$$

Thus

$$\Re e \left\{ \frac{D^{n+1}f(z)}{D^n f(z)} - (p+1) \right\} \geq \left\{ \frac{2\lambda(p-\alpha)k-4\alpha(1+(p-\alpha))}{(1+(2p-2\alpha+1)w(z_0))(1+w(z_0))} \right\} > 0, \quad (2.8)$$

which contradicts (2.1). Hence $|w(z)| < 1$ and from (2.3) $f \in M(n, \lambda, \alpha, p)$.

Corollary (1)

All $f \in M(n, \lambda, \alpha, p)$ belong to $\Sigma(\beta)$ for all n ,

$$\text{where } \beta = \frac{p-\alpha+\lambda}{\lambda}.$$

In the next theorem we derive some properties of the operator D^n and give an application of the following Miller-Mocanu lemma [3].

Lemma 2.2 [3]

Let $\Phi(u, v)$ be a complex valued function, $\Phi : D - \mathcal{E}, D \subset C \times C$ (C is a complex plane), and

let $u = u_1 + iu_2$ and $v = v_1 + iv_2$. Suppose that the function $\Phi(u, v)$ satisfies the conditions

- (i) $\Phi(u, v)$ is continuous in D ;
- (ii) $(1, 0) \in D$, $\Re e \{ \Phi(u, v) \} > 0$;
- (iii) $\Re e \{ \Phi(iu_2, v_1) \} \leq 0$; for all $(iu_2, v_1) \in D$

such that $v_1 \leq \frac{-(1+u_2^2)}{2}$. If $h(z) = 1$

+ $c_n z^n + \dots$, is an analytic function in U and $\Phi(h, zh'; z) \in D$ for all $z \in U$, then $\Re e h(z) > 0$.

Theorem 2.2

If $f \in M(n+1, \lambda, \alpha, p)$, then $f \in M(n, \lambda, \beta, p)$ for β where

$$\beta = \frac{2(\alpha-p-1) + \sqrt{4(\alpha-p)^2 - 12(\alpha-p) + 17}}{4}.$$

Proof

Let us define by

$$\left\{ \frac{D^n f(z)}{D^{n-1} f(z)} - (p+1) \right\} = -\{\beta + (p-\beta)h(z)\}, \quad (2.9)$$

$$\text{with, } \beta = \frac{2(\alpha-p-1) + \sqrt{4(\alpha-p)^2 - 12(\alpha-p) + 17}}{4}.$$

Then $h(z) = 1 + p_1 z + p_2 z^2 + \dots$ is analytic in the open disk U .

Differentiating (2.9) logarithmically, we obtain

$$\frac{\lambda z (D^n f(z))'}{D^n f(z)} = \frac{(z \lambda D^{n-1} f(z))'}{D^{n-1} f(z)} + \frac{-(p-\beta)\lambda z h'(z)}{(1+p-\beta) - (p-\beta)h(z)}.$$

Thus

$$\frac{D^{n+1} f(z)}{D^n f(z)} = \frac{D^n f(z)}{D^{n-1} f(z)} - \frac{(p-\beta)\lambda z h'(z)}{(1+p-\beta) - (p-\beta)h(z)}$$

and

$$\begin{aligned} & \Re e \left\{ -\frac{D^n f(z)}{D^{n-1} f(z)} + (p+1) - \beta \right\} \\ &= \Re e \left\{ -\frac{D^{n+1} f(z)}{D^n f(z)} + (p+1) - \beta + \frac{(p-\beta)\lambda zh'(z)}{[(\beta-p-1) + (p-\beta)h(z)]} \right\} \\ &= \Re e \left\{ (\alpha-\beta) + (p-\alpha)h(z) + \frac{(p-\beta)\lambda zh'(z)}{(\beta-1-p) + (p-\beta)h(z)} \right\} > 0. \end{aligned}$$

Let us define a function $\Phi(u, v)$ by

$$\Phi(u, v) = (\alpha - \beta) + (p - \alpha)u - \frac{(p - \beta)\lambda v}{[(1 + p - \beta) - (p - \beta)u]}.$$

Then we set

- (i) $\Phi(u, v)$ is continuous in $D = \left\{ C - \frac{p - \beta + 1}{(p - \beta + 1) - 1} \right\}$
- (ii) $(0, 1) \in D, \Re e \{ \Phi(0, 1) \} = p - \alpha > 0$
- (iii) For all $(iu_2, v_1) \in D$ such that $v_1 \leq -\frac{(1 + u_2^2)}{2}$

$$\begin{aligned} \Re e \{ \Phi(iu_2, v_1) \} &= \Re e \left\{ (\alpha - \beta) + (p - \alpha)iu_2 + \frac{(p - \beta)\lambda v_1}{[(\beta - p - 1) + (p - \beta)iu_2]} \right\} \\ &\leq (\alpha - \beta) - \frac{(\beta - p - 1)(p - \beta)\lambda(1 + u_2^2)}{2\{(\beta - p - 1)^2 + (p - \beta)^2 u_2^2\}} \leq 0. \end{aligned}$$

The function $\Phi(u, v)$ satisfies the conditions of Lemma 2.2. Consequently, we obtain

$$\Re e \left\{ \frac{D^n f(z)}{D^{n-1} f(z)} - (p - 1) \right\} < \frac{2(\alpha - p - 1) + \sqrt{4(\alpha - p)^2 - 12(\alpha - p) + 17}}{4}$$

Hence the theorem.

We now derive a sufficient condition for a function f to be in $M(n, \lambda, \alpha, p)$.

Theorem 2.3

If $f \in \Sigma_p$ in U and defined by (1.6) and

$$\sum_{k=1}^{\infty} [1 + (k - p - 1)\lambda]^n |\lambda(k - p - 1) - (p - \alpha)(1 - (p + 1)\lambda)| a_{k-p} \leq (p - \alpha) |1 - \lambda(p + 1)|^{n+1}, \quad (2.10)$$

then $f \in M(n, \lambda, \beta, p)$.

Proof

Suppose (2.10) holds for all admissible values of n, λ, p and α . Using the hypotheses, a simple calculation shows that for all $z \in U$ we have

$$\begin{aligned} & \left| 2(p - \alpha) + 1 - \frac{D^{n+1} f(z)}{D^n f(z)} - \left| \frac{D^{n+1} f(z)}{D^n f(z)} - 1 \right| \leq \left| -2 \frac{D^{n+1} f(z)}{D^n f(z)} + 2(p - \alpha) + 2 \right| \right. \\ &= \left| (\alpha - p) + \sum_{k=1}^{\infty} \frac{[1 + (k - p - 1)\lambda]^n [\lambda(k - p - 1) - (1 - (p + 1)\lambda)(p - \alpha)]}{(1 - (p + 1)\lambda)^{n+1}} a_{k-p} z^k \right| \\ &\leq -(\alpha - p) + \frac{\sum_{k=1}^{\infty} [1 + (k - p - 1)\lambda]^n [\lambda(k - p - 1) - (p - \alpha)]}{(1 - (p + 1)\lambda)^{n+1}} |a_{k-p} z^k| \leq 0, \end{aligned}$$

by hypothesis. According to the maximum modulus theorem we get $f \in M(n, \lambda, \beta, p)$.

When $f \in T(n, \lambda, \alpha, p)$, then the above sufficient condition is also necessary and we have the following.

Theorem 2.4

Let f be defined by (1.8). Then $f \in T(n, \lambda, \alpha, p)$ if and only if

$$\sum_{k=1}^{\infty} [1 + (k - p - 1)\lambda]^n \left| \frac{[\lambda(k - p - 1) - (p - \alpha)(1 - (p + 1)\lambda)]}{(1 - (p + 1)\lambda)^{n+1}} \right| a_{k-p} \leq (p - \alpha) \quad (2.11)$$

Proof

Here, we only need to prove the “if part”. Let $f \in T(n, \lambda, \alpha, p)$. Then

$$\left| \frac{-\frac{D^{n+1}f(z)}{D^n f(z)} + 2(p-\alpha)+1}{\frac{D^{n+1}f(z)}{D^n f(z)} - 1} \right| < 1. \quad (2.12)$$

Using the fact that $\Re e\{z\} \leq |z|$ for all z , we have the following

$$\Re e \left\{ \frac{(p-\alpha) - \sum_{k=-p+1}^{\infty} \frac{[2(p-\alpha)(1-(p+1)\lambda) - (k-1)\lambda]}{(1-(p+1)\lambda)^{n+1}} (1+(k-1)\lambda)^n a_k z^k}{-\sum_{k=-p+1}^{\infty} \left[\frac{1+(k-1)\lambda}{1-(p+1)\lambda} \right]^n [(k-1)\lambda] a_k z^k} \right\} < 1.$$

Let us choose values of z on the real axis so that $\frac{D^{n+1}f(z)}{D^n f(z)}$ is real. Upon clearing the denominator in (2.12) and letting $z \rightarrow 1^-$ through the real values, we obtain the required condition.

The function

$$f_k(z) = \frac{1}{z^p} - \frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+(k-p-1)\lambda]^n [\lambda(k-p-1) - (p-\alpha)(1-(p+1)\lambda)]} z^{k-p}, k \geq 1, \quad (2.13)$$

is an extremal function for the theorem.

Corollary (2)

If $f \in T(n, \lambda, \alpha, p)$, then

$$a_k \leq \frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+(k-1)\lambda]^n [\lambda(k-1) - (p-\alpha)(1+(p+1)\lambda)]},$$

for each $k = -p+1, -p+2, -p+3, \dots$

The equality holds for the function given by (2.13).

Corollary (3)

$$T(n+1, \lambda, \alpha, p) \subseteq T(n, \lambda, \alpha, p), \text{ for all } n \subseteq \mathbb{N}.$$

3. Closure theorems**Theorem 3.1**

The class $T(n, \lambda, \alpha, p)$ is closed under convex combinations.

Proof

Let $f, g \in T(n, \lambda, \alpha, p)$ and let f is given by (1.8) and

$$g(z) = \frac{1}{z^p} - \sum_{k=-p+1}^{\infty} b_k z^k, b_k \geq 0$$

For $0 \leq \delta \leq 1$, it is sufficient that the function h defined by

$$h(z) = (1-\delta)f(z) + \delta g(z), (z \in U)$$

belongs to $T(n, \lambda, \alpha, p)$. Since

$$h(z) = \frac{1}{z^p} - \sum_{k=-p+1}^{\infty} [(1-\delta)a_k + \delta b_k] z^k$$

applying the Theorem 2.4, we get

$$\begin{aligned} & (1-\delta) \sum_{k=1}^{\infty} [1+(k-p-1)\lambda]^n [\lambda(k-p-1) - (1-(p+1)\lambda)(p-\alpha)] a_{k-p} \\ & + \delta \sum_{k=-p+1}^{\infty} [1+(k-p-1)\lambda]^n [\lambda(k-p-1) - (p-\alpha)(1-(p+1)\lambda)] b_{k-p} \\ & \leq [(1-\delta)(p-\alpha) + \delta(p-\delta)] (1-(p+1)\lambda)^{n+1} \\ & = (p-\delta)(1-(p+1)\lambda)^{n+1}, \end{aligned}$$

implies $h \in T(n, \lambda, \alpha, p)$.

From Theorem 3.1 it follows that the closed convex hull of $T(n, \lambda, \alpha, p)$ is the same as $T(n, \lambda, \alpha, p)$. Now we determine the extreme points of $T(n, \lambda, \alpha, p)$.

Theorem 3.2

Let $f_{-1}(z) = \frac{1}{z^p}, f_k(z) = \frac{1}{z^p} - \frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p-1)]^n[\lambda(k-p-1)-(p-\alpha)(1-(p+1)\lambda)]} z^{k-p}$,

where $k=1,2,3,\dots,z \in U$ and $n \in \mathbb{N}_0$. Then $f \in T(n,\lambda, \alpha, p)$ if and only if it can be expressed as

$$f(z) = \sum_{k=-p}^{\infty} \sigma_k z^k$$

where $\sigma_k \geq 0$, and $\sum_{k=-p}^{\infty} \sigma_k = 1$.

Proof

Suppose that

$$f(z) = \sum_{k=-p}^{\infty} \sigma_k f_k(z) = \frac{1}{z^p} - \sum_{k=1}^{\infty} \sigma_k \left(\frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p-1)]^n[\lambda(k-p-1)-(p-\alpha)(1-(p+1)\lambda)]} \right) z^{k-p}$$

Since

$$\sum_{k=-p}^{\infty} \frac{[1+(k-1)\lambda]^n[\lambda(k-1)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \sigma_k \left(\frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+(k-1)\lambda]^n[\lambda(k-1)-(p-\alpha)(1-(p+1)\lambda)]} \right) = \sum_{k=-p}^{\infty} \sigma_k - 1 - \sigma_{-p} \leq 1,$$

it follows from the Theorem 2.4 that $f \in T(n,\lambda, \alpha, p)$.

Conversely suppose that

$$f(z) = \frac{1}{z^p} - \sum_{k=-p+1}^{\infty} a_k z^k \in T(n,\lambda, \alpha, p),$$

and since

$$a_k \leq \frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+(k-1)\lambda]^n[\lambda(k-1)-(p-\alpha)(1-(p+1)\lambda)]}, k = -p+1, -p+2, \dots,$$

we set

$$\sigma_k = \frac{[1+\lambda(k-1)]^n[\lambda(k-1)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} a_k \text{ for } k = -p+1, -p+2, \dots$$

and

$$\sigma_{-p} = 1 - \sum_{k=-p+1}^{\infty} \sigma_k.$$

From Theorem 2.4, we have

$$\sum_{k=-p}^{\infty} \sigma_k \leq 1, \text{ and } \sigma_{-p} \geq 0.$$

It follows that

$$f(z) = \sum_{k=-p}^{\infty} \sigma_k f_k(z).$$

Corollary (4)

The extreme points of $T(n,\lambda, \alpha, p)$ are the function $f_k, k = 1,2,3,\dots$ where f_k is given by (2.3).

4. Partial sums of $p(n,\lambda,\alpha,p)$ and $t(n,\lambda,\alpha,p)$

Following the earlier works by Silverman [5] on the partial sums of analytic functions, we study the ratio of a function of the form (1.8) to its sequence of partial sums of the form

$$f_n(z) = \frac{1}{z^p} - \sum_{k=-p+1}^{-p+n} a_k z^k, \tag{4.1}$$

where the coefficients of f satisfy the condition (2.1). We also determine sharp lower bounds for

$$\Re e \left\{ \frac{f(z)}{f_n(z)} \right\}, \Re e \left\{ \frac{f_n(z)}{f(z)} \right\}, \Re e \left\{ \frac{f'(z)}{f'_n(z)} \right\} \text{ and } \Re e \left\{ \frac{f'_n(z)}{f'(z)} \right\}.$$

It is seen that this study not only gives, as a particular case, the results of Silverman [6] but also gives rise to several new results.

Theorem 4.1

If f is of the form (1.8) and satisfies the condition (2.11), and

$$\frac{[1+(m-p)\lambda]^n}{(1-(p+1)\lambda)^{n+1}} [\lambda(m-p)-(p-\alpha)(1-(p+1)\lambda)] \geq \begin{cases} (p-\alpha) & m=1,2,\dots,k \\ \frac{[1+\lambda(m-p)]^n}{(1-(p+1)\lambda)^{n+1}} [\lambda(m-p)+(p-\alpha)(1-(p+1)\lambda)] & m=k+1,k+2,k+3,\dots \end{cases}$$

then for ($z \in U$)

$$\Re \left\{ \frac{f(z)}{f_k(z)} \right\} \geq \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)] - (p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]} \quad (4.2)$$

$$\Re \left\{ \frac{f_k(z)}{f(z)} \right\} \geq \frac{[1+\lambda(p-\alpha)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1} + [1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]} \quad (4.3)$$

The results (4.2) and (4.3) are sharp for a function given by (2.13).

Proof

Let us define the function w by

$$\frac{1+w(z)}{1-w(z)} = \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}}$$

$$\times \left[\frac{f(z)}{f_k(z)} \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)] - (1-(p+1)\lambda)^{n+1} (p-\alpha)}{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]} \right]$$

$$= \frac{1 + \sum_{j=1}^k a_{j-p} z^j + \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} a_{j-p} z^j}{1 + \sum_{j=1}^k a_{j-p} z^j} \quad (4.4)$$

It is sufficient to show that $|w(z)| \leq 1$. From (4.4) we can write

$$w(z) = \frac{\left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} a_{j-p} z^j}{2 + 2 \sum_{j=1}^k a_{j-p} z^j + \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} a_{j-p} z^j}$$

and

$$|w(z)| \leq \frac{\left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} |a_{j-p}|}{2 - 2 \sum_{j=1}^k |a_{j-p}| - \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} |a_{j-p}|}$$

Now $|w(z)| \leq 1$ if

$$2 \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} |a_{j-p}| \leq 2 - 2 \sum_{j=1}^k |a_{j-p}|$$

or equivalently

$$\sum_{j=1}^k |a_{j-p}| + \sum_{j=k+1}^{\infty} \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) |a_{j-p}| \leq 1 \quad (4.5)$$

From the condition of (2.10), it is sufficient to show that

$$\sum_{j=1}^{\infty} |a_{j-k}| + \sum_{j=k+1}^{\infty} \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}|$$

$$\leq \sum_{j=1}^{\infty} \frac{[1+\lambda(j-p-1)]^n [\lambda(j-p-1)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}|$$

which is equivalent to

$$\sum_{j=1}^k \frac{[1+\lambda(j-p-1)]^n [\lambda(j-p-1)-(p-\alpha)(1-(p+1)\lambda)]-(p-\alpha)(1-(p+1)\lambda)^{n+1}}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}| +$$

$$\sum_{j=k+1}^{\infty} \frac{[1+\lambda(j-p-1)]^n [\lambda(j-p-1)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}|$$

$$- \sum_{j=k+1}^{\infty} \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}| \geq 0. (4.6)$$

To see that the function given by (2.13) shows that this result is sharp, let

$$f(z) = \frac{1}{z^p} + \frac{(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]} z^{k-p}, k=1,2,\dots$$

$$= \frac{1}{z^p} - \frac{(\alpha-p)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]} z^{k-p}$$

$$\frac{f(z)}{f_k(z)} = \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]-(p-\alpha)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}$$

when $r \rightarrow 1^-$.

To prove the second part of this theorem, we write

$$\frac{1+w(z)}{1-w(z)} = \frac{(p-\alpha)(1-(p+1)\lambda)^{n+1} + [1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}}$$

$$\times \left[\frac{f_k(z)}{f(z)} - \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1} + [1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]} \right]$$

$$= \frac{1 + \sum_{j=-p+1}^{k-p} a_j z^{j+p} - \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k-p+1}^{\infty} a_j z^{j+p}}{1 + \sum_{j=-p+1}^{k-p} a_j z^{j+p}},$$

where

$$|w(z)| \leq \frac{\left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k-p+1}^{\infty} |a_j|}{2 - 2 \sum_{j=-p+1}^{k-p} |a_j| - \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k-p+1}^{\infty} |a_j|} \leq 1.$$

This last inequality is equivalent to

$$\sum_{j=-p+1}^{-p+k} |a_j| + \sum_{j=k-p+1}^{\infty} \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_j| \leq 1.$$

Making use of (2.1) we get (4.3). Finally, equality holds in (4.3) for the extremal function f given by (2.13).

Remark 4.1

Different choices of λ, n, p and α give the above result for many well known classes.

Corollary (5)

Let $\lambda = 1, n = 0$ in Theorem 2.1. $f \in \sum_p^*(\alpha)$ is given by (1.6) and

$$\sum_{k=1}^{\infty} [k-p+(p-\alpha)p] |a_{k-p}| \leq p(\alpha-p).$$

Then for $z \in U$

$$\Re e \left\{ \frac{f(z)}{f_k(z)} \right\} \geq \frac{(k-p+(p-\alpha)p)+(p-\alpha)p}{(k-p+(p-\alpha)p)}$$

and

$$\Re e \left\{ \frac{f_k(z)}{f(z)} \right\} \geq \frac{(k-p+(p-\alpha)p)}{-p(p-\alpha)+(k-p+(p-\alpha)p)}.$$

This result is sharp for

$$f_1(z) = \frac{1}{z^p} + \frac{(p-\alpha)(p)}{(k-p+(p-\alpha)p)} z^{k-p}, k=1,2,3,\dots \quad (4.7)$$

Corollary (6)

Let $\lambda = 1$, $n = 1$, $f \in \Sigma_k(\alpha)$ and if it satisfies

$$\sum_{k=1}^{\infty} [k-p+1][k-p+(p-\alpha)p] |a_{k-p}| \leq p^2(\alpha-p).$$

then for $z \in U$

$$\Re e \left\{ \frac{f(z)}{f_k(z)} \right\} \geq \frac{(k-p+1)(k-p+(p-\alpha)p)+p^2(p-\alpha)}{(k+1-p)(k-p+(p-\alpha)p)}$$

and

$$\Re e \left\{ \frac{f_k(z)}{f(z)} \right\} \geq \frac{(k+1-p)(k-p+(p-\alpha)p)}{(p-\alpha)p^2+(k+1-p)(k-p+(p-\alpha)p)}.$$

The result is sharp for the function given by

$$f_k(z) = \frac{1}{z^p} - \frac{(p-\alpha)p^2}{(1+k-p)(k-p+(p-\alpha)p)} z^{k-p}. \quad (4.8)$$

Theorem 4.2

If f of the form (1.6) satisfies the condition (2.1), and

$$\frac{[1+\lambda(m-p)]^n [\lambda(m-p)-(1-(p+1)\lambda)(p-\alpha)] \geq$$

$$\left\{ \begin{array}{ll} m(p-\alpha) & m=1,2,3,\dots \\ m[1+\lambda(k-p)]^n \left[\frac{\lambda(m-p)-(p-\alpha)}{k+1} \right] & m=k+1, k+2,\dots \end{array} \right.$$

then

$$\Re e \left\{ \frac{f'(z)}{f'_k(z)} \right\} \geq \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)] - (p-\alpha)(k-p+1)(1-(p+1)\lambda)^{n+1}}{[1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}, \quad (4.9)$$

and

$$\Re e \left\{ \frac{f'_k(z)}{f'(z)} \right\} \geq \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(1-(p+1)\lambda)(p-\alpha)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}(k-p+1) + [1+\lambda(k-p)]^n [\lambda(k-p)-(p-\alpha)(1-(p+1)\lambda)]}, \quad (4.10)$$

where $z \in U$ and $k = 1, 2, \dots$

Proof

We write

$$\frac{1+w(z)}{1-w(z)} = \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}}$$

$$\times \left[\frac{f'(z)}{f'_k(z)} - \frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(1-(p+1)\lambda)(p-\alpha)] - (k-p+1)(1-(p+1)\lambda)^{n+1}(p-\alpha)}{[1+\lambda(k-p)]^n [\lambda(k-p)-(1-(p+1)\lambda)(p-\alpha)]} \right],$$

where

$$w(z) = \frac{\left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=1-p}^{\infty} ja_j z^{j+p}}{2+2 \sum_{j=1-p}^{k-p} ja_j z^{j+p} + \left(\frac{[1+\lambda(k-p)]^n [\lambda(k-p)-(1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k-p+1}^{\infty} ja_j z^{j+p}}$$

Now $|w(z)| \leq 1$ if

$$\sum_{j=1}^{k-p} j |a_j| + \left(\frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (p-\alpha)(1-(p+1)\lambda)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k-p+1}^{\infty} j |a_j| \leq 1.$$

Now it is sufficient to show that

$$\sum_{j=1}^k (j-p) |a_{j-p}| + \left(\frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} (j-p) |a_{j-p}|$$

$$\leq \sum_{j=1}^{\infty} \frac{[1 + \lambda(j-p-1)]^n [\lambda(j-p-1) - (1-(p+1)\lambda)(p-\alpha)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}|,$$

that is

$$\begin{aligned} & \sum_{j=1}^k \frac{[1 + \lambda(j-p-1)]^n [\lambda(j-p-1) - (p-\alpha)(1-(p+1)\lambda)] - (p-\alpha)(1-(p+1)\lambda)^{n+1} (j-p)}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}| \\ & + \sum_{j=k+1}^{\infty} \frac{(j-p+1)[1 + \lambda(j-p-1)]^n [\lambda(j-p-1) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}| \\ & - \sum_{j=k+1}^{\infty} \frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (p-\alpha)(1-(p+1)\lambda)](j-p+1)}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}| \geq 0. \end{aligned}$$

To prove (4.10), let us define w by

$$\frac{1+w(z)}{1-w(z)} = \frac{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1} + [1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}}$$

$$\times \left[\frac{f'_k(z)}{f'(z)} - \frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1} + [1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]} \right],$$

where

$$w(z) = \frac{\left(1 + \frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} (j-p) a_{j-p} z^j}{2 + 2 \sum_{j=1}^k (j-p) a_{j-p} z^j + \left(1 + \frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} (j-p) a_{j-p} z^j}$$

Now $|w(z)| \leq 1$, if

$$\sum_{j=1}^k (j-p) |a_{j-p}|$$

$$+ \left(1 + \frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) \sum_{j=k+1}^{\infty} (j-p) |a_{j-p}| \leq 1.$$

It is sufficient to show that the left side is bounded by

$$\sum_{j=1}^{\infty} \frac{[1 + \lambda(j-p-1)]^n [\lambda(k-p-1) - (p-\alpha)(1-(p+1)\lambda)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} |a_{j-p}|,$$

which is equivalent to

$$\begin{aligned} & \sum_{j=1}^k \left(\frac{[1 + \lambda(j-p-1)]^n [\lambda(k-p-1) - (1-(p+1)\lambda)(p-\alpha)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} - (j-p) \right) |a_{j-p}| \\ & + \sum_{j=k+1}^{\infty} \left(\frac{[1 + \lambda(k-p-1)]^n [\lambda(k-p-1) - (1-(p+1)\lambda)(p-\alpha)]}{(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) |a_{j-p}| \\ & - \sum_{j=k+1}^{\infty} \left(1 + \frac{[1 + \lambda(k-p)]^n [\lambda(k-p) - (1-(p+1)\lambda)(p-\alpha)]}{(k-p+1)(p-\alpha)(1-(p+1)\lambda)^{n+1}} \right) (j-p) |a_{j-p}| \geq 0. \end{aligned}$$

Remark 4.2

Different choices of λ, n, p , give the above result for many well known classes of meromorphic functions. For example

Corollary (7)

Let f be given by (1.6) and if it satisfies the condition

$$\sum_{j=1}^{\infty} [j-p-1 + (p-\alpha)p] |a_{j-p}| \leq (\alpha-p)p$$

then $f \in \Sigma_p(\alpha)$ and for $z \in U$

$$\Re \left\{ \frac{f'(z)}{f'_n(z)} \right\} \geq \frac{[n-p+(p-\alpha)p] + (p-\alpha)(n-p+1)p}{(n-p+(p-\alpha)p)}, \quad n=1,2,3,4,\dots$$

and

$$\Re \left\{ \frac{f'_n(z)}{f'(z)} \right\} \geq \frac{(n-p+(p-\alpha)p)}{(\alpha-p)p(n-p+1) + (n-p+(p-\alpha)p)}, \quad n=1,2,3,\dots$$

In both cases the extremal function is given by (4.7).

Corollary (8)

Let f be of the form (1.6) and satisfy the condition

$$\sum_{k=1}^{\infty} (k-p)(k-p-1+(p-\alpha)p) |a_{k-j}| \leq p^2(p-\alpha), \quad k=1,2,3,\dots$$

Then $f \in \Sigma_k(\alpha)$ and for $z \in U$

$$\Re \left\{ \frac{f'(z)}{f'_k(z)} \right\} \geq \frac{(k-p+(p-\alpha)p) - p^2(p-\alpha)}{(k-p+(p-\alpha)p)}, \quad k=1,2,3,\dots$$

and

$$\Re \left\{ \frac{f'_k(z)}{f'(z)} \right\} \geq \frac{(k-p+(p-\alpha)p)}{(p-\alpha)p^2 - (k-p+(p-\alpha)p)}, \quad k=1,2,3,\dots$$

In both cases the extremal function is given by (4.8).

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Review

BARLEY GENETICS AND GENOMICS: A REVIEW

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Received October 2005, accepted January 2006

Communicated by Prof. Dr. Khushnood A. Siddiqui

Summary: This article discusses some recent developments in the use of DNA molecular markers in barley. The progress made in DNA marker technology has been tremendous and exciting. These markers have provided valuable tools in various analyses ranging from phylogenetic analysis to the positional cloning of genes. The importance of barley as a crop plant has prompted widespread genetic research onto this species. Genetic and physiological studies have been used to scan the components of primary gene pool of barley (wild, landraces and modern cultivars) for tolerance to various abiotic stresses. Landraces and particularly, wild barley genotypes exhibit huge variation of interest to breeders in improving the modern crop for specific traits. The introgression of this exotic variation into modern barley cultivars has been problematic in the past due to linkage drag (associated deleterious genes), difficulties in recognizing and testing for the presence of introgressed characters and background genetic effects. Molecular marker procedures now provide a means of overcoming these problems. This review provides an overview of current developments and looks into the application of these technologies for barley breeding.

Introduction

The genus *Hordeum* belongs to the tribe Triticeae, in the grass family Poaceae, and comprises two subspecies: *spontaneum* and *agriocrithon*. *H. spontaneum* is an annual plant with a short life cycle, diploid with only seven pairs of chromosomes, and self-pollinating. The genetic diversity of *H. spontaneum* has been identified by many markers, including isozyme polymorphisms [1,2] RFLP-markers [3,4] RAPD-markers [5] SSR-markers [6,7], AFLP-markers [8,9] and SNP-markers [10] respectively. *H. spontaneum* possesses more variation than cultivated barley, and many alleles are associated with specific environments [11,12]. Distinct geographic patterns of genetic diversity are maintained in wild barley (*H. spontaneum*) despite migration [13].

Conscious selection of desired genotypes by farmers at an early stage, together with natural selection, increased the diversity and created the rich gene pool, the source of variation found today in local varieties. These landraces also formed the basic material for modern plant breeding, which started about 150 years ago [14].

With the development of malting and brewing industries, barley became the major source of raw material. Barley is favored by temperate conditions of climate, and some of the best malting grain is consequently produced in districts bordering the sea coast, [15]. Meanwhile, barley production has increased significantly as the demand for livestock feed has grown. Also, barley is a special-purpose grain rather than a general market crop, finding its greatest use as a substitute for maize in animal feeding and for malting. Consequently, from the

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middle of the twentieth century, barley occupied the fourth position in the world's cereal acreage, following the larger acreages of wheat, rice, and maize. (see Table 1) [16].

Fischbeck [17,18] estimated that nearly 85 % of the current world barley production is used for feeding animals, and most of the rest for malting industry. Consequently, barley is transformed into the human food supply system, indirectly. In the area of EU, feed domestic consumption rate was 70% in 2002 [19]. Moreover, the need for malting barley as a major material for the brewing industry should be taken in consideration while the total world beer production increases steadily. Europeans produce 25% of the total world beer production, which is equal to 320 million hectoliters of beer each year (The Brewers of Europe). The famous German beer industries should be mentioned with its over thousand brewing plants owning 100 million hectoliters production ability, leading to the importance of malting barley in German cereal production. For these reasons, the focus of breeding is not only increasing the yield of barley production but also improvement of the malting barley quality.

Contribution of wild barley to crop improvement

Domestication and selection have resulted in a drastic narrowing of the genetic base of crop

species [20] including barley [21]. In recent years, breeding for uniformity has accelerated this process and has led to greater susceptibility of crops to diseases, pests, and abiotic stresses [22]. The genetic bottlenecks arising from the transitions between wild genotypes to early domesticated germplasm, and from early domesticated germplasm to modern cultivars has left behind many potential useful genes. Until the late 19th century, all cultivated barley existed as landraces. Some landraces persist to the present day, especially in developing countries, through selection and breeding has largely replaced landraces with pure line cultivars.

In conventional plant breeding, new varieties are generated from a primary adapted pool of elite germplasm. In the past decades, intensive breeding of crop varieties has further narrowed the gene pool, especially in self-pollinated crops [23]. Due to limited genetic variation among modern crops, efficient use of the genetic variation available in unadapted or wild relatives of modern cultivars is therefore necessary for continued improvement of cereal varieties [20]. In Europe the barley disease powdery mildew (*Erysiphe graminis*) that causes yields losses as high as 50 percent [24] forces breeders to exploit wild species.

The wild progenitor of cultivated barley, *H. spontaneum* has contributed many useful genes

Table 1.
World cereal acreage (million hectare).

Year	Wheat		Rice		Maize		Barley		Sorghum	Millet	Oat	Rye	Triticale	Total
	Acre	%	Acre	%	Acre	%	Acre	%	Acre	Acre	Acre	Acre	Acre	Acre
1982	238.5	33.38	141.8	19.85	124.4	17.40	77.90	10.90	45.60	35.60	25.60	17.30	0.69	714.6
1987	220.6	31.62	141.3	20.26	129.9	18.63	78.1	11.19	46.0	34.0	23.0	16.3	1.27	697.5
1992	222.4	31.37	147.3	20.77	137.0	19.32	73.5	10.37	46.3	37.5	19.5	14.7	2.43	709.1
1997	226.3	32.34	151.0	21.58	141.3	20.19	63.4	9.07	45.1	36.2	15.5	10.8	2.60	699.7
2002	210.6	32.00	147.1	22.35	138.8	21.08	52.2	7.92	42.6	33.4	13.5	9.5	3.07	658.2

Source: [16]

for several characters, especially diseases resistance to powdery mildew [25] and leaf rust [26]. Many agronomic traits were investigated in this species, such as yield and its components [27,28,29] floral structure [30] protein content [31] spikelet weight [32] stem and spike length [33]. Variation in physiological traits associated with salt tolerance [34] cold tolerance [35] drought tolerance and N-starvation [36] has also been studied in *H. spontaneum*. Therefore, *H. spontaneum* is not only a rich source of new disease resistance, but also an important species to offer genetic variability for economically important traits.

The generation of modern elite cultivars is a process based on decades of selections by breeders. The productivity, uniformity and quality of these cultivars are obvious differences from those of wild or unadapted germplasm. Therefore, once wild species carrying undesirable genes were applied into breeding plan, the negative effects followed with these genes - linkage drag - will be a considerable problem. In the past, breeding to introduce polygenic characters into a balanced population from wild species has been generally avoided. In order to make improvements of crops with unlimited resources of wild species and unadapted germplasm, it is necessary to find out an approach to reduce or break the linkage drags.

Barley is an inbreeding species and single plant selection, which promotes uniformity, has been common since the 1800s. The wild progenitor species and the primitive landraces of barley offer rich sources of genetic variation for crop improvement [27,37]. These gene pools can be exploited using conventional breeding procedures but with the aid of genetic maps, markers and quantitative trait locations (QTL analysis) greater precision can be obtained in selecting desirable genotypes.

Molecular mapping of the barley genome

Genetic mapping in barley

New approaches, especially trisomic analysis has been successfully used in the chromosomal mapping of barley [38]. Barley (*Hordeum vulgare* L.) has an excellent system for genome mapping and map-based analyses [39] because its chromosomes are homoeologous to cultivated wheat and rye, respectively [40]. Similarly, barley is an established model species for genetic and physiological studies [41]. The cytology and genetics of barley showed that it is a diploid ($2n = 2x = 14$), self-pollinated species. Seven barley chromosomes were identified and labeled based on their sizes and characteristics [42]. Chromosomes 1 through 5 differ in their sizes measured at mitotic metaphase, with chromosome 1 being the longest and chromosome 5 being the shortest; chromosomes 6 and 7 have satellites, with chromosome 6 having the larger satellite and chromosome 7 having the smaller satellite [43]. Since the barley chromosomes have the same DNA content as those in other members of the Triticeae, and the gene loci in barley are largely collinear with the loci in other members of the Triticeae, with few ancestral translocations involving whole chromosome segments, chromosomes 1 to 7 of barley (*Hordeum vulgare* L.) were redesignated as chromosomes 7H, 2H, 3H, 4H, 1H, 6H, and 5H respectively [44,45]. The barley genome present in the variety 'Betzes' became the reference genome in the barley to which definitions of translocations and, short arm/long arm reversals were standard in all species. Meanwhile, wheat barley chromosome addition lines were available for 'Betzes', so other Triticeae workers have an incentive to test their probes on barley [46].

Cytogenetic methods such as translocation analysis and the primary trisomic method were

introduced in the early 1950s and greatly contributed to the establishment of cytogenetic linkage maps [47]. More than 60 isozyme markers were detected in barley [48,49,50] and a well-developed classical genomic map was constructed for barley using isozyme and morphological markers [51].

Molecular markers

RFLP markers

The development of restriction fragment length polymorphism (RFLP) for high density genomic mapping in human [52] provided a new technique which overcame some of the problems associated with isozymes and proteins [53,54]. Since then, RFLP markers have been widely used to construct linkage maps for several crop species, including maize [55] rice [56] and tomato [57]. Restriction endonucleases are enzymes that cleave DNA molecules at specific nucleotide sequences depending on the particular enzyme used. Since genomic DNA differs in nucleotide sequences, fragments of different sizes may be generated for different plant accessions when digested with restriction endonucleases and separated by gel electrophoresis. The fragmented DNA can be transferred from agarose gels to Nylon filters by Southern blotting. By hybridization with cDNA probes or other cloned single- or low copy DNA elements labeled radioactively, fragments of different sizes are observed on Nylon filters containing digested DNA by autoradiography. Polymorphic cDNA probes and other cloned single- or low-copy DNA elements are called RFLP-markers.

The first application of RFLP genetic mapping in barley was on chromosome 6H by Kleinhofs *et al.* [58] followed by [59,60,61,62]. This technique has been a powerful tool for barley in comparative mapping studies among species

of triticeae [63,64] resulting in the construction of a consensus map [65] and gene mapping [66,67]

RAPD markers

PCR (Polymerase Chain Reaction) has revolutionized molecular genetics. The development of a PCR-based arbitrarily primed genetic assay called RAPD (Random Amplified Polymorphic DNA), [68] AP-PCR [69] or DAF (DNA Amplification Fingerprinting, [70] has been widely used for the construction of genetic maps [71,72,73,74,75] and has greatly changed the prospects for application of molecular markers to study populations and to accelerate breeding [76,77]. In particular, RAPD markers provide a very powerful tool to generate relatively dense linkage maps in a short period of time. Amplification products of the RAPD assay are specific DNA fragments with arbitrarily 10-base oligonucleotides as primer. Polymorphisms detected between individuals presumably result from numerous changes including sequence differences in one or both of the primer binding sites, insertion/deletion events or rearrangement in priming sites or in the internal amplified sequence and are defined by the presence or absence of a particular amplified product [78,79]. Thus, the arbitrarily primed PCR products are usually dominant markers and cannot distinguish homozygous and heterozygous states.

Compared with the RFLPs, the advantage of the arbitrarily primed PCR techniques such as RAPDs were, the requirement of small amounts of DNA (5-20ng), the rapidity to screen for polymorphisms, the efficiency to generate a large number of markers for genomic mapping and the potential automation of the technique [80]. (Since the first two reports about detection and mapping of the RAPD markers in barley by [5,81] RAPD analysis as a simple and easy-to-handle method was used for tagging of genes, such as genes for

resistance to barley blotch [82,83] and barley yellow dwarf virus [84,85].

AFLP markers

Amplified Fragment Length Polymorphism (AFLP) as a PCR-based fingerprinting technique was first described by Zerbe and Vos [86]. The AFLPTM technology is under patent owned by KeyGene N.V. (www.keygene.com). It is based on the selective amplification of a subset of genomic restriction fragments using PCR [87]. Genomic DNA is digested with restriction endonucleases and ligated to synthetic adaptors. Thus, the sequence of the adaptors and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments by PCR. Selective nucleotides extending into the restriction fragments are added to the 3' ends of the PCR primers such that only a subset of the restriction fragments are recognized. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides are amplified. The subset of amplified fragments is then analyzed by denaturing polyacrylamide gel electrophoresis to generate the fingerprint. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously, however, is dependent on the resolution of the detection system. The level of polymorphism is species specific. Compared with allozymes, RAPDs, and RFLPs, AFLPs have superior performance in the time and cost efficiency, replicability and resolution, except that the AFLP method primarily generates dominant rather than co-dominant markers [88].

Since the first report about AFLP mapping in barley was published [89] several maps of AFLP makers have been constructed [39, 90, 91, 92]. Later, it became an important tool in barley

genetic research, including the investigation of the origin of barley [93, 94] diversity studies [95, 96,97,98] mapping of QTL [8,99,100,101,102, 103,104,105] detection of disease resistance genes [105,106] and fine mapping of disease resistance genes such as *mlo* [107] *Mla* [108 and *Rph15* [109] .

STS markers

An STS (Sequence Tagged Sites) [110] is a unique, single copy segment of the genome whose DNA sequence is known by sequencing and which can be amplified by specific PCR. With a set of primers of about 20-25 nucleotides in length derived from a stretch of DNA with a known sequence, unique DNA segments of about 90-300 bps can be amplified. A combination of advantages (markers are PCR based, no clone maintenance or distribution is necessary) and their co-dominant mode of inheritance make STS markers an important marker system in crop plants [77,111,112]. The main advantage of STS markers lies in the speed with which they can be analyzed once PCR primer pairs have been identified.

Following the first reported mapping of STS markers in barley [113] a large amount of RFLP markers was converted to STSs for cultivar fingerprinting purpose [114] for physical mapping [115] and for mapping specific genes [116,117,118]. Later, a genetic map covered the entire barley genome [119] and new sources for development of STS maker in barley were available from EST sequencing results [120].

SSR markers

Simple sequence repeats (SSRs) [121] also called microsatellites, are stretches of DNA consisting of tandemly repeated short units of 1-6 basepairs in length, and are codominantly

inherited [122]. Such motifs are abundant and highly polymorphic in the genome of eukaryotes [123]. Microsatellites can be found anywhere in the genome, both in protein-coding and noncoding regions. The conserved sequences in the flanking regions of simple sequence repeats can be designed as a pair of specific primers to detect the DNA length polymorphism via the polymerase chain reaction [124,125]. A high level of polymorphism is to be expected because of the proposed mechanism responsible for generating SSR allelic diversity by replication slippage [126]. The SSR markers can be identified by sequencing microsatellite-containing clones isolated from small-insert genomic DNA libraries via hybridization with synthetic oligonucleotide probes, a method which is time-consuming and relatively expensive. A low cost way of SSRs development is screening of sequences in the public database.

The most frequently found repetitive motifs of mono-, di-, tri-, or tetranucleotide units are (A)_n, (GA)_n, (TAT)_n and (GATA)_n in plants [127]. The most abundant dimeric microsatellite in several well-known mammals is the AC repeat [128] while in many plant species they are AT or GA repeat [129]. More than 75% of the barley genome comprises repetitive DNA sequences [130]. It is estimated that the barley genome contains one GA repeat every 330kb and one GT repeat every 620kb [131] which is in agreement to the findings that GA repeats occur in barley at a higher frequency than GT repeats by Struss and Plieske [132]. Similar results were obtained with other important crops, such as wheat [133,134], rice [135] and maize [136]. Among trinucleotide repeats in barley, (CCG)_n, (AGG)_n and (AGC)_n repeats are the most-frequent motifs while (ACGT)_n and (ACAT)_n in tetrameric microsatellites [137].

The discovery of microsatellites has significantly increased the marker density of

linkage maps for some mammals, human [138,139] and mouse [140]. Molecular linkage maps in many model plants and crops were improved rapidly by the addition of SSR markers, such as in *Arabidopsis* [141] rice [142] wheat [143] and maize [144]. The informative value of microsatellite markers for genetic studies and as a powerful tool for barley breeding was confirmed in several studies [131,132,145, 146]. Among several important DNA marker systems, SSR markers showed the highest polymorphism, followed by RFLPs, RAPDs and AFLPs [97]. A second-generation linkage map of barley using only PCR-based microsatellite markers was constructed [147]. Besides microsatellites derived from genomic clones, also ESTs were exploited for the development of PCR-based SSR-markers [137,148,149].

SNP markers

The most general type of polymorphism, known as single nucleotide polymorphism (SNP), results from a single base mutation which substitutes one base for another. Other types of genetic polymorphisms result from the insertion or deletion of a section of DNA, which include microsatellite repeat sequences and gross genetic losses and rearrangements. Polymorphisms can be caused by mutations ranging from a single nucleotide base change to variations in several hundred bases. The mining of SNPs involving non gel-based assays and has recently been facilitated by the availability of genome-wide sequences and EST databases. A genetic map of the human genome was constructed showing the location of 2227 SNPs [150]. Scientists have identified about 1.4 million locations where single-base DNA differences (SNPs) occur in humans. The polymorphisms of SNP loci also were characterized in many other plants, such as rice [151] beet [152] maize [153] and soybean [154]. Many SNPs have been found with

screening entire genome sequences in *Arabidopsis thaliana* (The Arabidopsis Genome Initiative 2000) and *Oryza sativa* [155,156]. For many larger genomes, SNPs can be detected with scanning their EST sequences. For barley, SNPs have been successfully developed and applied in genetics studies [10,157,158,159]. Due to their abundance and slow mutation rate within generations, they are thought to be the next generation of genetic markers that can be used in a myriad of important biological, genetic, pharmacological, and medical applications. A high-resolution map of barley will be published in the near future, containing 1,044 loci and including 611 RFLP loci, 190 SSR loci and 255 SNP loci.

Genetic diversity in wild barley has also been studied using RFLPs [3] RAPDs [5] Simple sequence repeats [6] and AFLPs [8]. The wild barley used in the AFLP study has been tested for responses to a number of abiotic stresses including, drought, salinity, N-starvation, cold, ozone and day length.

QTLs in barley

Mapping of agronomic traits

For over a decade, with development of molecular approaches, QTL analysis was used to detect yield and fecundity-related traits. The most important agronomic trait in all crops of economic importance and in barley is yield, which is very complex. Many QTLs affecting yield were mapped on seven chromosomes throughout the whole genome. As summarized in Table 2, different numbers of QTL for yield were detected in different populations and environmental conditions. However, many of them are very difficult to be validated. A six-row cross, Steptoe \times Morex (SM), has been extensively developed as mapping population. Based on the phenotypic data from sixteen locations, 14 QTLs for yield were mapped on seven chromosomes in this mapping population [160]. Of them, only five on 2H, 3H, 5H, and 6H were confirmed in the same cross by Romagosa *et al.* [161,162] and Han *et al.* [163], respectively.

Table 2.
Major agronomic traits identified by QTL analyses in barley.

Agronomic trait	Chromosome	Reference
Yield	1H, 2H, 3H, 4H, 5H, 6H, 7H	160, 161, 162, 214, 229, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241
Grain number per ear	1H, 2H, 3H, 4, 5H, 6H	214, 229
Grain weight per year	1H, 2H, 3H, 4H, 5H, 6H, 7H	229, 237, 242
Grain weight per plant	1H, 2H, 3H, 4H, 5H, 6H, 7H	229
Thousand grain weight	2H, 3H, 4H, 5H, 6H, 7H	40, 214, 229, 234, 235
Ear breaking	2H, 4H, 6H	232
Ear length	2H	235
Spike number	3H	240, 244
Root length	5H	245
Plant height	1H, 2H, 3H, 4H, 5H, 6H, 7H	40, 91, 99, 160, 214, 234, 237, 238, 239, 246, 247
Heading date	1H, 2H, 3H, 4H, 5H, 6H, 7H	91, 99, 160, 214, 232, 234, 237, 238, 239, 247
Kernel weight	1H, 2H, 4H, 5H	172, 234
Kernel length	4H, 7H	172, 173, 232
Kernel plumpness	1H, 2H, 4H, 5H, 7H	172, 173
Kernel shape	1H	232
Dwarfing	2H, 3H, 4H	241, 248

The other two agronomic traits, heading date and plant height were easier to be investigated and were evaluated as additional important information to total yield.

Mapping of malting quality

The improvement of malting quality also is an important objective for barley breeders because of its major industry usage in brewing. However, malting quality is a complex character related to many traits, such as malt extract percentage, total grain protein, soluble protein, ratio of soluble/total protein, β -glucan content, kernel plumpness, α -amylase activity, diastatic power, and malt β -glucanase [164]. The malting process involves the interactions of a number of genes expressed during seed germination and development, depending on the temperature required during the reaction process [165]. Not only yield and its components are affected by genetic factors and environments, malting qualities of barley are also influenced by these factors. The enzymes β -amylase and α -amylase

convert gelatinized starch and glucans into sugars [166,167]. Five QTLs for malting quality were detected around the amylase loci on 2H, 4H and 6H [160] which was the first report for QTL analysis for malting quality. Han *et al.* [168] (1995) mapped 12 loci for β glucan content in barley grain and activity of β -glucanase that degrades cell wall β -glucan in malting process, respectively. Summarizing many data over years and locations, a region on chromosome 7H near the centromere was identified to be a complex QTL region controlling malt extract, α -amylase activity, diastatic power and β -glucanase, etc. [169]. Higher protein content and ratio of soluble/total protein affect the quality of products and increase the cost of production. Though storage and structural protein (*GluA*, *GluB* and *Glu.c1*) were mapped on 1H [61] QTLs for protein content and ratio were mapped on seven chromosomes [170,171,172,173]. Moreover, a large number of QTL for seed dormancy and germination were reported by Han *et al.* [163,174] Thomas *et al.* [175] Romagosa *et al.* [176] and Gao *et al.* [177] (see Table-3). Of them,

Table 3.
Main traits for malting quality in barley and chromosomal location.

Malting trait	Chromosome	Reference
α -amylase	1H, 2H, 4H, 5H, 6H, 7H	160, 167, 170, 173, 216, 217, 218, 219.
β -amylase	2H, 4H	160, 220, 221
β -glucan	1H, 3H, 4H, 5H, 7H	168, 172, 218, 219
α -glucosidase	7H	222, 223
β -glucanase	1H, 2H, 4H, 5H, 7H	224, 2125, 226, 227
Diastatic Power	1H, 2H, 3H, 4H, 5H, 6H, 7H	99, 160, 170, 171, 172, 173, 175, 218, 219
Fermentability	2H, 3H, 5H	228
Grain Nitrogen	1H, 2H, 3H, 4H, 5H, 6H, 7H	229, 230
Grain Protein	1H, 2H, 3H, 4H, 5H, 6H, 7H	160, 170, 171, 172, 173
Sol./Total Protein	1H, 2H, 3H, 4H, 5H	170, 172, 173
Hot water extract	1H, 2H, 3H, 4H, 5H, 6H	99, 175, 230
Malt extract	1H, 2H, 4H, 5H, 6H, 7H	160, 170, 173, 219
Seed dormancy	5H	174, 176, 177
Germination	2H, 3H, 5H, 6H, 7H	175, 231

a major dormancy locus on chromosome 5H was verified in different labs.

Mapping disease resistance gene

The conclusion that on average yield losses of 10.5% in barley are caused by diseases is based on 15,700 literature references and 3,700 field trials [178]. Jørgensen [179] published a list of 83 loci rendering resistances to important barley diseases. Graner [180] provided a valuable review of molecular mapping of qualitative and quantitative disease resistance genes. Current state of resistance study and breeding in barley were summarized in detail by Kleinhofs and Han [43], Chelkowski *et al.* [181] and Weibull *et al.* [182]. Barley growth is mainly damaged by fungal diseases and virus diseases (see Table-4). Fungal diseases comprise powdery mildew, scald, rust diseases (leaf rust, stripe rust and stem rust), net

blotch and others. Barley is attacked by several viruses, which are the barley yellow dwarf viruses, the cereal yellow dwarf virus, the barley stripe mosaic virus, the barley yellow streak mosaic virus, and the wheat dwarf virus.

Table 5.
Summary of Barley QTL report [169].

Trait	Abiotic Stress resistance	Agronomic traits	Biotic stress resistance	Quality traits	Other
# of phenotypes measured	26	58	15	27	5
# of populations	7	16	10	8	3
# of QTL	67	389	103	180	18

Summary of reported QTL in barley (see Table-5), 757 QTL covers the whole barley genome for abiotic stress resistance, agronomic traits, biotic stress resistance, quality traits and others [169].

Table 4.

Major resistance genes against 15 fungal pathogens, four virus and two pests in barley [181,182].

Gene Symbol	Pathogen, Pest & Disease	# of Genes
Rph	<i>Puccinia hordi</i> (leaf rust)	17
Rpg	<i>Puccinia graminis</i> (stem rust)	4
Rps	<i>Puccinia striiformis</i> f.sp. <i>hordi</i>	4
Ml (Mlo, Mla, MILa & Reg)	<i>Erysiphe graminis</i> f. sp. <i>hordi</i> (powdery mildew)	23
Rcs	<i>Cochliobolus sativus</i> (spot blotch)	5
Rpt	<i>Pyrenophora teres</i> (net blotch)	6
Rdg (Rhg)	<i>Pyrenophora graminea</i> (barley stripe)	3
Rrs (Rh)	<i>Rhynchosporium secalis</i> (scald)	14
Run (un)	<i>Ustilago nuda</i> (loose smut)	8
Ung	<i>Ustilago nigra</i> (semiloose smut)	1
Ruh	<i>Ustilago hordei</i> (covered smut)	4
Rsp	<i>Ustilago hordei</i> (covered smut)	3
Rti	<i>Typhula incarnate</i> (gray snow mold)	1
Fb	<i>Fusarium</i> spp. (scab)	1
Ryd	BYDV (barley yellow dwarf virus)	2
Rym	BaYMV (barley yellow mosaic virus)	13
	BaMMV (barley mild mosaic virus)	
Rsm	BSMV (barley stripe mosaic virus)	5
Rsg	<i>Schizaphis graminum</i> (green bug aphid)	3
Rha	<i>Heterodera avenae</i> (cereal cyst nematode)	3

Advanced backcross-QTL analysis

Eshed and Zamir [183] proposed to use variations of the backcross method [184] combined with genetic map information to map QTLs and select families with desired chromosomal regions. With the development of the molecular markers and linkage map, advanced backcross (AB) QTL mapping strategy [23] can be utilized to evaluate donor introgressions in the genetic background of an elite recurrent parent. Using this approach, favorable alleles and potentially valuable QTLs derived from either wild or adapted sources of germplasm and tagged with molecular markers can be associated with the performance of segregating offspring. In parallel, these QTL alleles will be transferred into near-isogenic lines (NILs) by means of marker associated selection breeding. Therefore, unlike the conventional QTL mapping methods, AB-QTL analysis can accelerate the process of marker based breeding because the end products of analysis are close to NILs carrying favourable alleles.

Since the 1980s, Tanksley *et al.* [185] has conducted genetic studies for fruit-size/shape and mapped 28 QTL of interesting traits using seven wild species of tomato and seven different crossing designs [186]. In Tanksley's lab, Alpert *et al.* [187] reported a major QTL, *fw2.2*, controlling fruit weight that was found in a wild tomato species with a BC1 population of 257 plants. In the next year, a favourable QTL allele (*fw9.1*) from the wild species was identified on chromosome 9, which increased fruit size by nearly 14% [185]. This BC1 population also was used to construct a genetic linkage map suitable for quantitative trait locus (QTL) analysis to be conducted in different backcross [188]. High resolution mapping and isolation of a YAC containing the site of *fw2.2* were accomplished [189]. Besides controlling the size of the

developing tomato fruit, *fw2.2* also had secondary effects on fruit number and photosynthate distribution as a negative fruit-growth regulator [190,191]. This was one of the first molecular characterizations of a locus that was originally identified entirely by QTL mapping, a landmark in QTL analysis. Moreover, *fs8.1*, a major QTL from wild species influencing fruit shape was characterized with the same population [192]. Its effect that could be traced with advanced backcross population (BC4F3) was identified to affect the fruit shape early in carpel development at least 6 days before anthesis with a set of NILs [193]. At the same time, some NILs segregating for this region of interest were obtained. In other crossings of wild tomato species to cultivated tomato, hundreds of QTL were detected over different locations for 19 agronomic traits, including for tomato flavor [194,195,196,197]. A larger number of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles were developed [198,199,200] and analyzed [201,202,203].

Since the first report in tomato [185], AB-QTL analysis has been successfully applied in many crops to detect and transfer valuable QTLs from unadapted germplasm into elite breeding lines, such as in rice [204,205,206,207,208,209,210,211], and in maize [212] (Ho *et al.* 2002). Recently, the first two AB-QTL studies in wheat and barley were published by [213] and [214], respectively.

Concluding Remarks

Future research activities aimed at identifying candidate genes for QTLs will increasingly rely on the contribution of the technological platforms of high throughput genotyping, microarrays, metabolic profiling etc. These will provide greater insight into the genomic make up (e.g. single nucleotide polymorphism, SNP,

haplotype at target regions) and the changes in the molecular and biochemical profiling of the plant brought about by drought and other environmental stresses [215]. The utilization of near isogenic lines at target QTLs coupled with the information provided by linkage disequilibrium studies will improve our capacity to resolve the genetic basis of crop production under drought and provide routes to cloning genes underlying major QTLs. Although QTL cloning remains daunting, particularly in cereals, the availability of recombinant chromosome substitution lines, contiged genomic libraries and sequence information will facilitate the process in future.

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